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Articles for publication in the Journal must bear the formal approval of the chief of the department bureau, or of the director of the experiment station from which the paper emanates. Each manuscript must be accompanied by a statement that it has been read and approved by one or more persons (named) familiar with the subject. The data as represented by tables, graphs, summaries, and conclusions must be approved from the statistical viewpoint by someone (named) competent to judge. All computations should be verified.

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CONTENTS

ERRATA AND AUTHOR'S EMENDATIONS

Page 3, column 6, "$(Y)^2$" should be "(Y)."

Page 7, 11th line from bottom, "left-hand" should be "upper" and "right-hand" should be "lower."

Page 14, figure 2, legend, D and E, "$\times 20$" should be "$\times 80$."

Page 24, line 11, "(p. 502)" should be "(p. 26)."

Page 26, 12th line from bottom, *"hibenalis"* should be *"hibernalis."*

Page 43, paragraph 1, line 3, "(Kell. and Sw. Magn.)" should be "(Kell. and Sw.) Magn.)."

Page 59, last line, "x²" should be "x^2."

Page 68, line 1, "80° C." should be "8° C."

Page 89, paragraph 1, line 4, "soil-improving crop" should be "soiling crop."

Page 219, paragraph 1, line 5, "077" should be "on."

Page 222, legend of figure 1, "H_9Cl_2" should be "H_gCl_2."

Page 297, last line, "3 or 4" should be "3 and 4."

Page 365, paragraph 1, line 3, "plants" should be "roots."

Page 405, paragraph 2, line 2, "Tuyes" should be "Teays."

INDEX

ESTIMATION OF CLEAN-FLEECE WEIGHT FROM GREASE-FLEECE WEIGHT AND STAPLE LENGTH [1]

By Clair E. Terrill, *animal husbandman*, Elroy M. Pohle, *associate animal fiber technologist*, L. Otis Emik, *junior animal husbandman*, and Lanoy N. Hazel, *assistant animal husbandman, Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

Clean-fleece weight is the most important measure of fleece value in breeding for improvement of sheep. This is particularly true within breeds when variations in grade and quality of wool are not great. Although most wool in the United States is sold on a grease-wool basis, the price is usually determined by the buyer's estimation of the amount of clean wool present. If this estimation is accurate, weight of clean wool is one of the most important factors affecting the income from wool.

The amount of clean wool per fleece can be determined exactly by scouring the entire fleece or approximately by scouring a representative sample of each fleece. However, even the latter practice requires much routine work when large numbers are involved, and adequate equipment and personnel are seldom available to carry on this routine continuously. This condition has prompted the search for methods that would make it possible to estimate clean-fleece weight from other fleece characters with a high degree of accuracy.

Correlations between various fleece characters of range Rambouillet sheep have been studied by Spencer and coworkers (*7, p. 45*).[2] They concluded:

> The scoured-fleece weights became greater as the weights became greater in the unscoured fleeces, and in the moisture, grease, and dirt per fleece. The length of staple was generally longer, the fineness a trifle less, and the character and density of the fleeces slightly more excellent as the yields of clean wool per fleece increased.

These writers showed that the weight of unscoured (grease) wool was the best factor for indicating the weight of scoured (clean) wool per fleece. Pohle and Keller (*4*), working with range Rambouillet, Targhee, Columbia, and Corriedale yearling ewes, found that with each centimeter (three-eighths inch) of increase in staple length there was an increase of about one-fourth to one-half pound, or almost 1 to 2.5 percent, of clean wool. Length of staple had a greater influence on clean-fleece weight than did body weight.

Lambert and coworkers (*2*) showed that fleece length in weanlings had some predictive value for estimating clean-fleece weight in yearlings in the Corriedale, Columbia, and Rambouillet breeds. Pohle (*3*) found in the same breeds that length of staple and percentage of clean wool in weanlings had a high relationship with these same characters in yearlings.

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[1] Received for publication July 8, 1943.
[2] Italic numbers in parentheses refer to Literature Cited, p. 10.

Previous work [3] with 186 Rambouillet ewes showed that fleece length and body weight at weanling or yearling ages were reliable in estimating clean-fleece weight in yearlings. The multiple correlation coefficients were 0.71 and 0.68 for weanling and yearling characters, respectively. Fleece length was more important than body weight in estimating clean-fleece weight, particularly in yearlings. The correlation coefficients of grease-fleece weight, fleece length, and body weight with clean-fleece weight were 0.69, 0.63, and 0.37, respectively, in yearlings. The multiple correlation coefficient between clean-fleece weight and the three variables was 0.786, as compared with 0.783 when body weight was omitted. Therefore, in yearlings, body weight did not add to the ability to estimate clean-fleece weight, probably owing to a fairly high relationship between body weight and grease-fleece weight. Other measures, such as fleece-character score, density score, density index, and fleece fineness at the side and thigh, had such low relationships with clean-fleece weight that they could have little value in its estimation.

The objects of this study, which involved several years' data from fairly large numbers of sheep of four breeds, were: (1) To develop multiple regression equations for the estimation of clean-fleece weight from grease-fleece weight and staple length; (2) to test the reliability of these equations; (3) to present a nomograph for the rapid estimation of clean-fleece weight; and (4) to develop formulas for annual corrections for variations in grease-fleece weight, staple length, and clean-wool yield. The work was conducted at the Western Sheep Breeding Laboratory and United States Sheep Experiment Station, Dubois, Idaho

MATERIAL AND METHODS

Data on grease-fleece weight, staple length, and clean-fleece weight from 1,037 Rambouillet, 253 Targhee, 214 Corriedale, and 211 Columbia yearling ewes were taken during the 3-year period from 1939 to 1941. These were fairly normal years with respect to clean-wool yield or shrinkage. In 1941 there were two groups of Rambouillet yearlings. Group A consisted of inbreds and group B of outbreds. The former animals were born a month earlier and were managed somewhat differently than the latter. The data for the two groups, therefore, are presented separately.

Just prior to shearing, staple length was measured near the middle of the side of each ewe to the nearest 0.2 cm., the average of three measurements by different judges being used as the actual length. Grease-fleece weight was taken on the shearing floor to the nearest 0.05 pound. Clean-wool yields, in terms of percentage, were determined by scouring a small sample of wool from each ewe. The small sample, weighing 25 to 35 gm., was obtained with the use of an electric clipper from an area about 11 cm. long and 5 cm. wide near the middle of the side. The samples were immediately placed in individual moisture-proof containers, being scoured later as described by Hardy (*1*) to obtain the bone-dry clean-wool yields. The term "bone-dry" refers to wool from which practically all the moisture had been eliminated by drying in a conditioning oven at 212° F. for a period of 6 hours. The clean-wool yield of the whole fleece was calculated from that of the small sample by the method described by Schott and coworkers (*5*).

[3] TERRILL, CLAIR E. Unpublished data.

The clean-fleece weight was then obtained by multiplying the grease-fleece weight by the calculated clean-wool yield of the whole fleece. Grease-fleece weights, staple lengths, and clean-fleece weights were taken at about 400 days' growth and were adjusted to 365 days' growth

$$\text{(adjusted measurement}=\frac{\text{actual measurement}}{\text{actual days growth}}\,365)$$

to minimize age differences.

Clean-fleece weights were also obtained from scoured whole fleeces of 85 Rambouillet, 46 Targhee, 55 Corriedale, and 55 Columbia yearling ewes. These fleeces were sent to the Agricultural Marketing Administration of the United States Department of Agriculture where they were scoured by the method described by Buck.[4]

DATA AND DISCUSSION

The general relationships among grease-fleece weight, staple length, and clean-fleece weight by breeds and years are shown by the averages, correlation coefficients, and standard partial regression coefficients in table 1. Correlations were highest between grease-fleece weight and clean-fleece weight and were lowest between grease-fleece weight and staple length. In every case grease-fleece weight was more important than staple length for the estimation of clean-fleece weight. However, staple length became progressively more important in the breeds with shorter staple and finer grades of wool.

TABLE 1.—*Average grease-fleece weight, staple length, and clean-fleece weight with correlation and standard partial regression coefficients for 4 breeds of sheep during a 3-year period*

Breed	Year data were taken	Sheep	Averages[1]			Correlation coefficients			Standard partial regression coefficients	
			Grease-fleece weight (X_1)	Staple length (X_2)	Clean-fleece weight $(Y)^2$	r_{Y1}	r_{Y2}	r_{12}	$\beta_{Y1.2}$	$\beta_{Y2.1}$
		Number	*Pounds*	*Centi-meters*	*Pounds*					
Rambouillet[3]	1939	195	8.29	5.65	2.77	0.78	0.57	0.40	0.65	0.31
	1940	272	9.11	5.68	3.09	.74	.67	.52	.53	.39
	1941A	308	9.33	5.95	3.42	.74	.63	.34	.59	.44
	1941B	262	8.96	5.67	3.26	.69	.62	.37	.54	.42
Average		1,037	8.98	5.75	3.17	.73	.63	.41	.57	.40
Targhee	1939	74	9.64	6.80	3.40	.73	.63	.41	.57	.39
	1940	91	10.33	7.34	4.07	.81	.62	.36	.67	.37
	1941	88	9.59	7.61	3.77	.79	.64	.45	.62	.36
Average		253	9.87	7.29	3.77	.78	.63	.41	.63	.37
Corriedale	1939	64	9.50	8.47	3.66	.72	.72	.46	.50	.49
	1940	72	9.38	8.36	3.83	.81	.61	.59	.69	.20
	1941	78	9.50	8.97	4.05	.83	.59	.35	.71	.35
Average		214	9.46	8.61	3.86	.79	.63	.46	.64	.34
Columbia	1939	61	9.86	7.89	3.78	.78	.59	.38	.65	.35
	1940	88	10.81	8.24	4.55	.77	.52	.30	.68	.32
	1941	62	10.61	8.59	4.27	.84	.59	.46	.72	.26
Average		211	10.47	8.24	4.24	.79	.56	.38	.68	.30

[1] All grease-fleece weights, staple lengths, and clean-fleece weights are for 365 days' growth.
[2] Clean-fleece weights are based on bone-dry yields.
[3] The two 1941 Rambouillet groups were kept separate because group A was inbred, born a month earlier, and managed somewhat differently than group B.

[4] BUCK, W. M. PROGRESS IN WOOL SHRINKAGE RESEARCH DURING YEAR 1939. U. S. Dept. Agr., Agr. Market. Serv. 32 pp. [Processed.] 1940.

Multiple regression and correlation coefficients for estimating clean-fleece weight from grease-fleece weight and staple length are given in table 2. The similarity of the multiple correlation coefficients, their range being from 0.80 to 0.89, indicates that the ability to estimate clean-fleece weight from the two factors did not vary much from year to year or from breed to breed.

TABLE 2.—*Multiple regression and correlation coefficients for estimating clean-fleece weight* (Y) *from grease-fleece weight* (X_1) *and staple length* (X_2)

Breed	Year data were taken	Regression coefficients			Correlation coefficient
		a	$b_{Y1.2}$	$b_{Y2.1}$	R
Rambouillet [1]	1939	−0.88	0.2644	0.2585	0.83
	1940	−.83	.2240	.3315	.81
	1941A	−1.14	.2519	.3712	.84
	1941B	−1.76	.2865	.4315	.80
Average within years		−1.10	.2513	.3500	.81
Targhee	1939	−1.78	.2792	.3665	.82
	1940	−1.74	.3391	.3124	.88
	1941	−1.42	.2934	.3118	.85
Average within years		−1.62	.3075	.3226	.85
Corriedale	1939	−1.98	.2573	.3770	.84
	1940	−.09	.2968	.1360	.83
	1941	−1.03	.3198	.2273	.89
Average within years		−.92	.2923	.2344	.85
Columbia	1939	−1.12	.2871	.2623	.84
	1940	−1.57	.3687	.2585	.83
	1941	−1.32	.3571	.2100	.87
Average within years		−1.35	.3420	.2442	.84

[1] The two 1941 Rambouillet groups were kept separate because group A was inbred, born a month earlier and managed somewhat differently than group B.

The significance of differences between the means of the characters studied was tested by analysis of variance, as shown in table 3. Breed differences were highly significant in each case, whereas yearly differences were not significant. The highly significant breeds-×-years interaction indicates that the breeds react differently to yearly environmental changes. The means for the two 1941 Rambouillet groups differed significantly in all three characters.

TABLE 3.—*Analysis of variance for grease-fleece weight, staple length, and clean-fleece weight*

Source of variance	Degrees of freedom	Grease-fleece weight		Staple length		Clean-fleece weight	
		Mean square	F [1]	Mean square	F [1]	Mean square	F [1]
Total	1,714	2.38		2.00		0.64	
Breeds [2]	3	158.91	11.49**	766.17	97.85**	91.96	10.69**
Years [2]	2	48.86	3.53	8.95	1.14	21.51	2.50
Breeds × years	6	13.83	6.92**	7.83	12.63**	8.60	20.00**
Rambouillet groups	1	17.57	8.79**	11.18	18.03**	3.59	8.35**
Error	1,702	2.00		.62		.43	

[1] ** $P<0.01$, highly significant.
[2] Mean squares tested with the breeds-×-years interaction.

Tests for significant differences of the a values are presented in table 4. These consist in comparing clean-fleece weights adjusted for average breed, yearly, or interaction variations in grease-fleece weight and staple length. The a values were significantly different for breeds, years, and breeds-$\times$-years interaction. Consequently, the a values in the multiple regression equation should be determined for each breed each year. The procedure for determining the a values is given later in this article in the application of results. The difference in average grease-fleece weight and staple length between the two 1941 Rambouillet groups accounted for practically all the difference in clean-fleece weight; hence, this test is not given in table 4.

TABLE 4.—*Tests for significant differences of a values*

Source of variance	Degrees of freedom	Sum of squares for clean-fleece weight (Sy^2)	R^2	Errors of estimate			
				Degrees of freedom	$Sy^2 - R^2 Sy^2$	Mean square	F [1]
Error	1,702	728.95	0.68264	1,700	231.34	0.1361	
Error+breeds	1,705	1,004.82	.76242	1,703	238.73		
Breeds				3	7.39	2.46	18.07**
Error+years	1,704	771.97	.67516	1,702	250.77		
Years				2	19.43	9.72	71.42**
Error+(breeds×years)	1,708	780.57	.69534	1,706	237.81		
Breeds×years				6	6.47	1.08	7.94**

[1] **P<0.01, highly significant.

Differences in the multiple regression coefficients were tested by an extension of the method given by Snedecor (*6*), as shown in table 5. Since the individual regressions for the two Rambouillet groups in 1941 were not significantly different, the average regressions ($b_{Y1\cdot2}$ and $b_{Y2\cdot1}$) were used for that year. The reduction in Sy^2 due to the average regression on grease-fleece weight or on staple length is shown in table 5. The additional reduction due to the four breed regressions was highly significant for both grease-weight and staple length, indicating that different regression coefficients should be used for each breed. Differences in yearly regressions were nearly significant, and those for the individual regressions for each breed each year were significant or highly significant. Breed differences in the regression coefficients were greater than yearly differences, but the breed regressions did not always occur in the same order. To estimate clean-fleece weight in succeeding years, it will be necessary to use the same regression coefficients each year. The above data indicate that these were not strictly homogeneous for the 3 years studied. However, the loss of information due to yearly variation in the actual regression coefficients is not likely to be large. In these data, errors of estimate of clean-fleece weight with the use of the average regression coefficients for each breed each year were 98.7 percent as large as the error of estimate with the use of the average regressions for each breed. Thus, the use of the four breed regression coefficients from year to year provides about 98.7 percent as much information as the calculation of new regression coefficients for each year. Likewise, the use of the average regression coefficients for all breeds and years provides about 97 percent as much information as the calculation of new regression coefficients for each breed each year. It seems worth while to use different

regression coefficients for each breed, as no additional work is required to estimate clean-fleece weights.

TABLE 5.—*Test of regression coefficients by years and breeds*

Source of variance	Degrees of free-dom	X_1 with X_2 fixed			X_2 with X_1 fixed		
		Sum of squares	Mean square	F^1	Sum of squares	Mean square	F^1
Error	1,702	728.95	--------	--------	728.95	--------	--------
Reduction due to other variable	12	279.94	--------	--------	420.94	--------	--------
Deviation from other variable	1,690	449.01	--------	--------	308.01	--------	--------
Reduction due to average regression	1	222.52	--------	--------	80.92	--------	--------
Additional reduction due to yearly regressions	2	.71	0.355	2.69	.79	0.395	2.99
Additional reduction due to breed regressions	3	1.67	.557	4.22**	1.54	.513	3.89**
Additional reduction due to individual regressions	6	2.14	.357	2.70*	2.79	.465	3.52**
Errors of estimate	1,678	221.97	.132	--------	221.97	.132	--------

[1] ** $P<0.01$, highly significant; * $P<0.05$, significant.

A practical test was made of the average within-year regression equations for each breed for estimating clean-fleece weight. Both small samples and whole fleeces of 241 yearling ewes were scoured, so that clean-fleece weight could be calculated in three ways for each breed: (1) By scouring the whole fleece (W); (2) by scouring a small sample (S); (3) by estimating from the within-year regression equations with grease-fleece weight and staple length as independent variables and S as the dependent variable (E).

The correlation coefficients between these variables are shown by breeds in table 6. Each of the multiple regression coefficients was calculated so the correlation between the dependent variable (S) and the estimated value (E) is a maximum. In all four breeds, values for r_{WE} were larger than those for r_{SE}, although the latter are similar to the multiple correlations given in table 2. This would be expected if the small samples were subject to unbiased errors, which would make S higher than the corresponding W in some cases and lower in others. The predictive value of grease-fleece weight and staple length for estimating clean-fleece weight, therefore, is higher than indicated by the multiple correlation coefficients involving these two factors and clean-fleece weight as determined from small samples.

TABLE 6.—*Correlation coefficients by breeds between clean-fleece weight, calculated by scouring the whole fleece (W), scouring a small sample (S), and estimating from grease-fleece weight and staple length (E)*

Breed	Fleeces	r_{WE}	r_{WS}	r_{SE}
	Number			
Rambouillet	85	0.86	0.89	0.80
Targhee	46	.89	.84	.82
Corriedale	55	.87	.91	.80
Columbia	55	.86	.94	.82
Average	--------	.87	.90	.81

In table 6 the average for r_{WE} was only slightly less than for r_{WS}, indicating that clean-fleece weight can be estimated almost as accurately from grease-fleece weight and staple length as from scouring a small sample.

APPLICATION OF RESULTS

The average within-year regression equations, obtained by the use of the average regression coefficients for each breed given in table 2, were used in constructing the nomograph presented in figure 1. Thus

YEARLING GREASE-FLEECE WEIGHT (POUNDS)

ESTIMATED CLEAN-FLEECE WEIGHT (POUNDS)
FOR
COLUMBIA

CORRIEDALE

TARGHEE

RAMBOUILLET

YEARLING STAPLE LENGTH AT SIDE (CENTIMETERS)
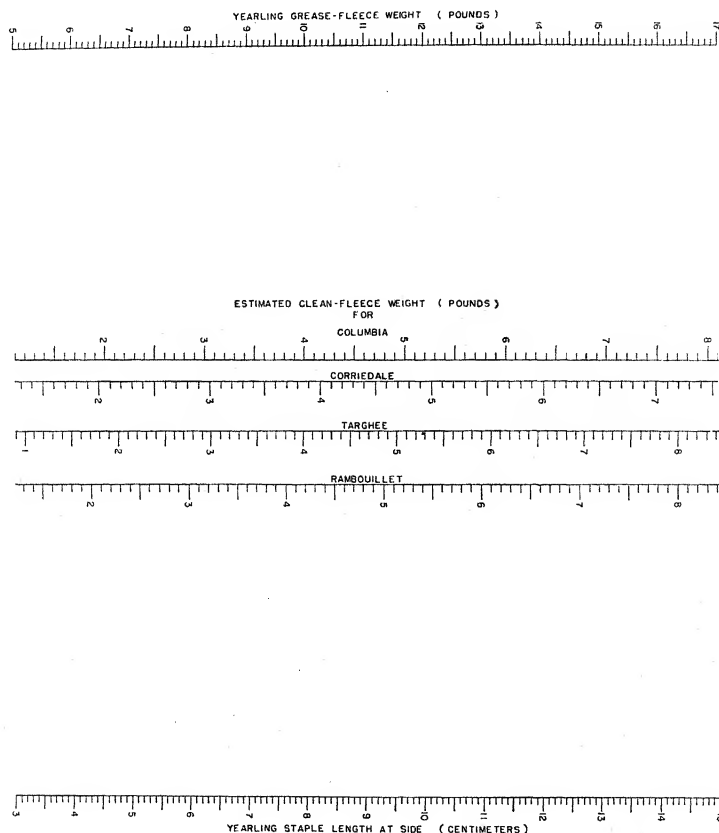
FIGURE 1.—A nomograph for the estimation of bone-dry clean-fleece weight from grease-fleece weight and staple length in four breeds of sheep. (Conversion may be made to an approximate commercial moisture basis by dividing the bone-dry weights by 0.88.)

by laying a ruler across the nomograph from a given grease-fleece weight on the left-hand scale to a given staple length on the right-hand scale, the estimated clean-fleece weight may be read directly from the middle scale for the respective breeds.

These clean-fleece weights will be adequate for use in a breeding program in which it is sufficient to rank individuals on clean-fleece weight each year. There will be yearly variations, however, in grease, dirt, moisture, and other foreign material that cannot be measured by a regression equation developed in previous years. Presumably variations of this kind were responsible for the significantly different *a* values as shown in table 4. In order to estimate clean-fleece weights that will be comparable from year to year and that will approach the

actual clean-fleece weights, it will be necessary to obtain the average percentage clean yields for each year. If small samples are scoured, percentage clean yield of whole fleeces (X) can be predicted from percentage clean yield of small samples (Y) by the simple regression equation $\frac{Y - a_{y \cdot x}}{b_{y \cdot x}} = X$, as shown by Schott and coworkers (5). With the same breeds used in this study, they found that $b_{y \cdot x} = 1.08$ could be used for each of the four breeds. The values of $a_{y \cdot x}$ were -2.86, -1.62, -0.84, and 0.73 percent for Rambouillets, Targhees, Corriedales, and Columbias, respectively, for small side samples. Then the average clean weight of a group of whole fleeces can be obtained by multiplying the estimated percentage clean yield of the whole fleeces $(\bar{X})$ by the average grease-fleece weight $(\bar{G})$.

Annual variations in average clean-fleece weight, grease-fleece weight, and staple length can be expressed as deviations from the a values used in the multiple regression equations so that annual corrections for the a values may be applied directly to the estimated clean-fleece weights obtained with the nomograph. Only one correction will need to be calculated for each breed each year. These corrections for the a values are as follows:

Rambouillet_____ $\left(\dfrac{\bar{Y} + 0.0286}{1.08}\right)\bar{G} - 0.2513G - 0.3500\bar{L} + 1.10$

Targhee_____ $\left(\dfrac{\bar{Y} + 0.0062}{1.08}\right)\bar{G} - 0.3075G - 0.3226\bar{L} + 1.62$

Corriedale_____ $\left(\dfrac{\bar{Y} + 0.0084}{1.08}\right)\bar{G} - 0.2923G - 0.2344\bar{L} + 0.92$

Columbia_____ $\left(\dfrac{\bar{Y} - 0.0073}{1.08}\right)\bar{G} - 0.3420G - 0.2442\bar{L} + 1.35$

where $\bar{Y}$ = average clean-wool yield from small side sample of a representative group of ewes, $\bar{G}$ = average grease-fleece weight for current year, and $\bar{L}$ = average staple length for current year.

For example, the correction for the a value for Corriedale yearling ewes in 1942 can be determined from the average clean-wool yield of 53.05 percent determined from small samples, the average grease-fleece weight of 7.9 pounds, and the average staple length of 10.1 cm. Thus the correction for the a value = $\left(\dfrac{0.5305 + 0.0084}{1.08}\right)(7.9) -$ $0.2923(7.9) - 0.2344(10.1) + 0.92 = 0.2$. Therefore, 0.2 pound should be added to the clean-fleece weight estimated from the nomograph for each Corriedale yearling ewe in 1942. A Corriedale yearling ewe in 1942 with a grease-fleece weight of 9.8 pounds and a staple length of 10.4 cm. would have an estimated clean-fleece weight of 4.4 pounds as determined from the nomograph. The corrected clean-fleece weight would be 4.6 pounds. All estimated clean-fleece weights would be for 365 days' growth since grease-fleece weight and staple length should first be adjusted to this basis. The clean-fleece weights obtained may be converted from bone-dry weights to a commercial moisture basis by dividing by 0.88.

The number of ewes from each breed that would need to be sampled for clean-fleece yield each year can be estimated from data presented by Schott and coworkers (5). If entire fleeces were scoured, a rea-

sonable degree of accuracy could be obtained by scouring fleeces from 19 ewes selected at random for each breed. The standard error for the mean whole-fleece yield would then be

$$0.0075 \left\{ = \sqrt{\frac{\overline{\sigma x^2}}{n}} = \sqrt{\frac{10.709}{19}} = 0.75 \text{ percent} \right\}.$$

The same degree of accuracy could be obtained by scouring a representative small sample from each of 30 ewes

$$\left\{ \sqrt{\frac{\sigma y^2}{(b_{y.x})^2(n)}} = \sqrt{\frac{19.449}{(1.1664)(30)}} = 0.75 \text{ percent} \right\}.$$

It should be remembered that the regression equations presented in this paper apply only where conditions are similar to those near Dubois, Idaho. Marked differences in climatic conditions, plane of nutrition, or other environmental factors may require different a and b values for the regression equations. Although the method outlined is general, the specific application of these equations can be tested only by developing similar equations for flocks in widely separated regions. Wool laboratories maintained in most of the important sheep-producing States can perform valuable service in this respect, thereby placing the selection of improved breeding stock and the marketing of grease wool on a more reliable basis than is possible where individual clean-fleece weights and average percentage clean yield are not known.

SUMMARY

Multiple regression equations of clean-fleece weight, as determined from small samples, on grease-fleece weight and length of staple (taken from middle of side) were calculated on 1,037 Rambouillet, 253 Targhee, 214 Corriedale, and 211 Columbia yearling ewes during the 3-year period from 1939 to 1941. Multiple correlation coefficients for these four breeds were 0.81, 0.85, 0.85, and 0.84, respectively.

Grease-fleece weight was more important than staple length for the estimation of clean-fleece weight. However, staple length became progressively more important in the breeds with shorter staple and finer grades of wool.

Analysis of variance was used to test for differences between means, a values, and the multiple regression coefficients. Breed differences were highly significant, but yearly differences were not significant for the means for each of the three characters. The a values were significantly different for breeds, years, and breeds×years, indicating that the a values should be determined for each breed each year. Significant differences were found in the regression coefficients for breeds but not for years.

The multiple regression equations were tested on data from 241 of the yearling ewes for which whole fleeces had been scoured. The within-year regression equations for each breed appeared to be somewhat more accurate than indicated by the multiple correlation coefficients for the larger group of data for which clean-fleece weights were calculated by scouring small side samples. The accuracy of grease-fleece weight and staple length for predicting clean-fleece weight was slightly less than that obtained by scouring a small sample.

A nomograph is presented for the rapid estimation of clean-fleece weight. Formulas are given for making annual corrections in the *a* values. These formulas account for annual variations in grease-fleece weight, staple length, and clean-wool yield as determined by scouring samples from a small group of ewes from each breed.

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RED STELE ROOT DISEASE OF THE STRAWBERRY CAUSED BY PHYTOPHTHORA FRAGARIAE[1]

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INTRODUCTION

The root disease of strawberries that has been described as Lanark-shire disease (*24*),[2] red core root (*1*), black stele (*4*), red stele,[3] and brown stele (*21*), first attracted attention in Lanarkshire, Scotland, about 1920 (*3, 24, 25*). The first published report of the occurrence of the disease in the United States was from Illinois in 1935 (*4*). Recent reports indicate that the disease has become widely disseminated in both the British Isles and the United States. According to the latest available references, its present distribution is as follows: In Scotland, 18 counties (*18*), including practically all strawberry-growing districts (*2*); in England, 5 counties (*18*), including Kent, Hampshire, Devon, and Cornwall (*11*); in the United States, Illinois (*4*), Maryland,[3] New Jersey,[3] New York,[3] Michigan,[3] Virginia,[3][4] Delaware,[5] Indiana,[6] Kentucky,[7] Connecticut,[8] Wisconsin,[9] California (*21*), Oregon,[10] and Massachusetts[11]; in addition, the authors have received specimens of the disease from Arkansas, Ohio, Pennsylvania, Tennessee, and Washington.

Investigations into the nature of the Lanarkshire disease were initiated soon after its discovery in Scotland. Wardlaw (*24, 25, 26*) attributed the disorder primarily to poor cultural conditions that paved the way for the entry of weak root parasites, and O'Brien and M'Naughton (*17*) to parasitism by endotrophic mycorrhiza. Alcock and her coworkers (*1, 2, 3*) first noted the association with diseased roots of oospores and sporangia that resembled those of *Phytophthora*, correctly deduced that this *Phytophthora* was the true cause of the disease, gave a brief description of it on its host plant, but did not name it; their attempts to isolate and grow the organism in pure culture were unsuccessful. Anderson (*4*) first reported the black stele

root disease of strawberries in the United States from Illinois and emphasized its similarity to the Lanarkshire disease. Bain and Demaree (*6*) isolated the fungus causing red stele root disease of strawberries in Maryland, emphasized its marked resemblance to the *Phytophthora* associated with red core root in Scotland (*1*), and regarded the fungus as probably representing a new species of *Phytophthora*. Thomas [12] isolated an apparently identical *Phytophthora* in California from the disease later (*21*) described as brown stele of strawberries. In 1938 Hickman (*10*) isolated the causal fungus of red core root from strawberries growing in Kent County, England, and in 1940 (*11*) he described it as a new species, *P. fragariae*. Hickman's article appeared as the present authors were concluding an extended study of the American red stele pathogen, here reported upon and shown to be identical with *P. fragariae* Hickman.

THE DISEASE

Above-ground symptoms of the red stele disease are most evident in the spring and early summer and usually are first noted as severe dwarfing and death of plants in the lower, poorly drained parts of the fields. Individual plants appear to decrease in size as the older outer leaves wilt, dry up, and are replaced by smaller new leaves borne on shortened petioles. A characteristic bluish-green color of leaves is often associated with dwarfed plants. On warm, dry days in early spring there may be a sudden wilting of entire plants or of the outer leaves only, followed by partial recovery at night or on cooler days. Severely affected plants either bear no fruit or they bear very little and that of decidedly inferior quality. Often entire fruit clusters dry up after setting normal complements of berries but before bringing any of them to maturity.

These symptoms in the aerial parts of the plant result from physiological drought and starvation, brought about by progressive destruction of the major part of the root system by the pathogen. Young lateral roots are often completely rotted by the *Phytophthora* or by secondary fungi and bacteria, so that when diseased plants are dug the long primary roots may come out of the soil conspicuously free from fibrous roots; these constitute a highly characteristic feature of the disease aptly described as "rat's-tail" roots (*2*). The fungus has not been observed in roots more than 1 year old or in the crown.

As the fungus advances upward into the older parts of fleshy roots, the central cylinder is killed first, and the *Phytophthora* mycelium may be wholly confined to these tissues. Coincident with penetration, the stele turns a brownish-red color, producing an effect that has given rise to most of the common names for the disease. This reddened stele, very conspicuous against the background of whitish nondiseased cortical tissue in roots stripped or cut open longitudinally, may extend several inches above the dead lower end of the root (fig. 1). Exterior tissues of the root die belatedly after the invasion of the stele, either as the result of delayed penetration by *Phytophthora* or some weaker parasite or merely as the result of cessation of the nutrient supply. The dead lower ends of infected roots often turn an inky-black color.

The annual course of the red stele disease begins with the late-fall dying of the tips of roots and ends with the coming of hot weather in

[12] Personal communication.

late spring or early summer.   During this period the roots which were first infected are gradually killed back to their origins at the crowns of the plants, while later infected roots may or may not be killed
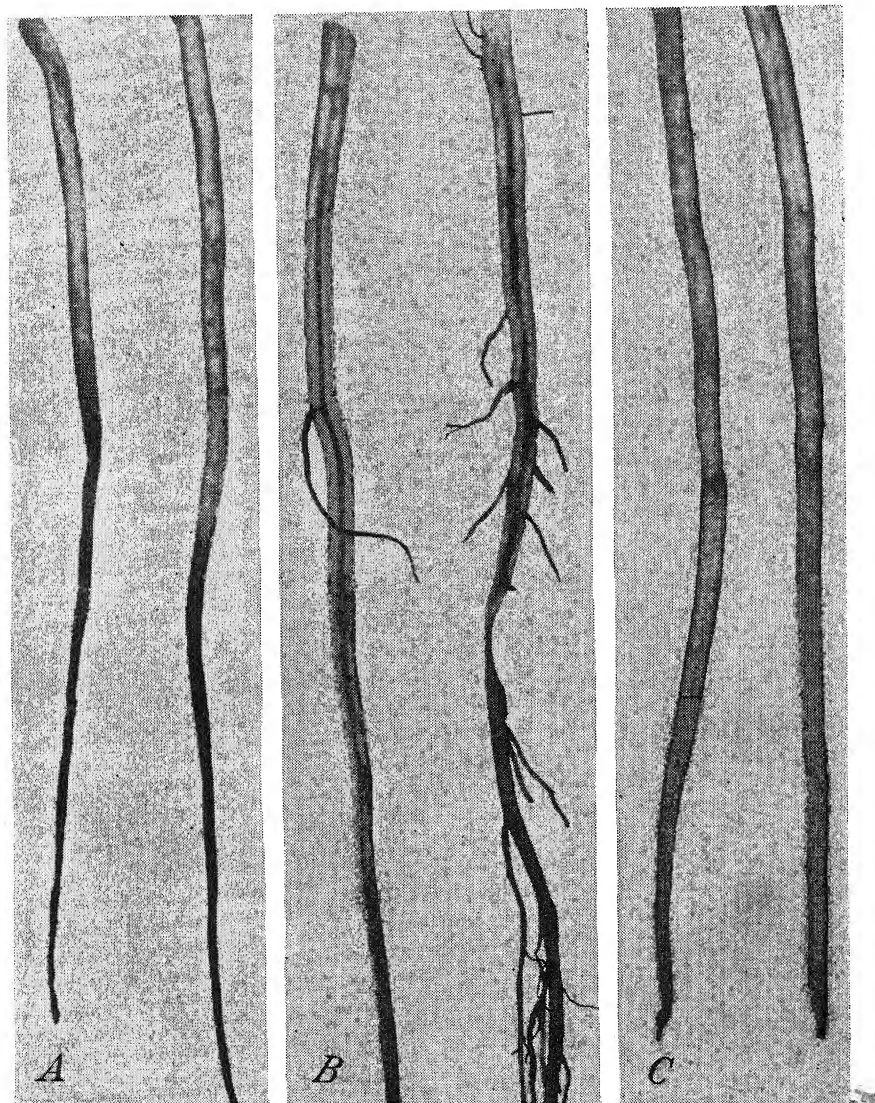
FIGURE 1.—Strawberry roots affected with the red stele disease.   *A*, Diseased roots showing dead, discolored tip ends; upper parts of roots not discolored. *B*, Diseased roots split lengthwise to show reddened central portion.   *C*, Normal roots split lengthwise to show absence of red center.

throughout their lengths before high temperature brings the disease to a complete stop.   If a sufficient number of roots escape infection through the cool weather of spring, the plant may recover to some extent during the summer and develop new roots that remain free
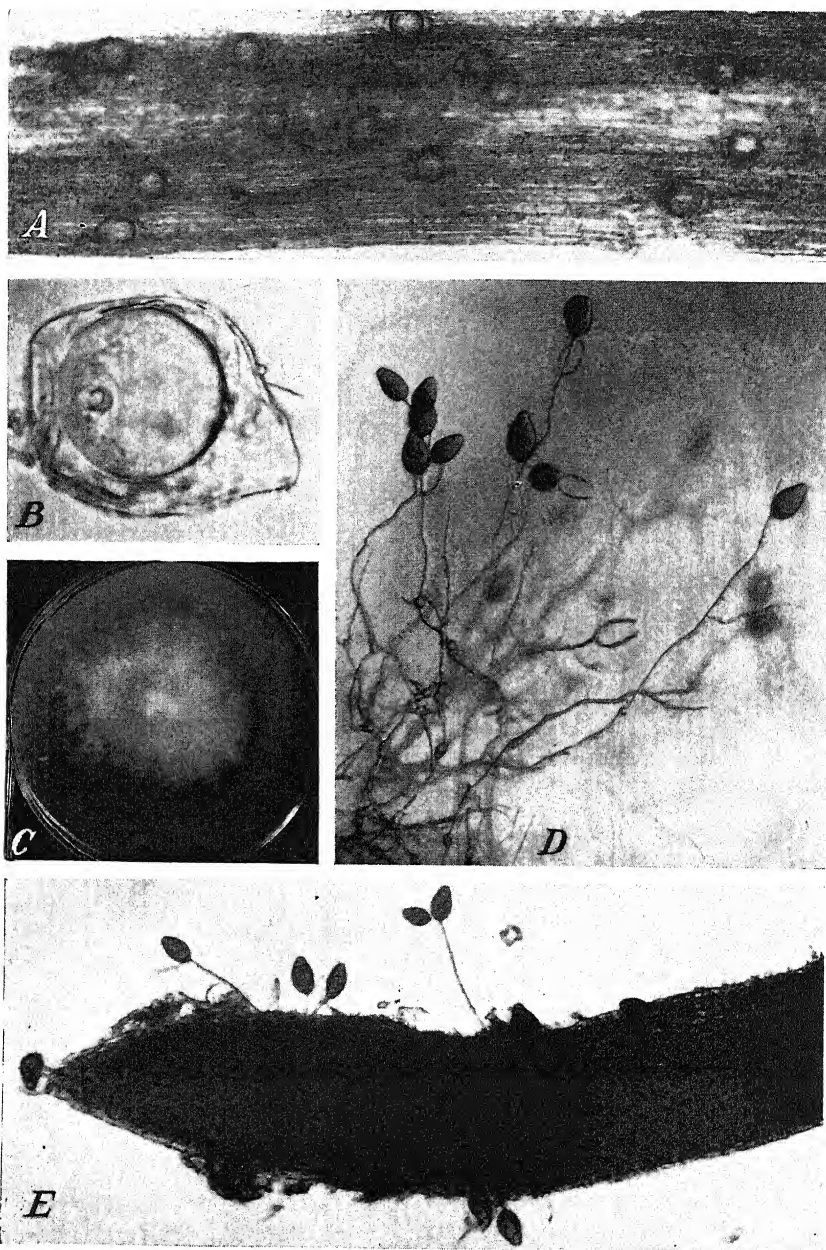
FIGURE 2.—*Phytophthora fragariae.* *A*, Oospores in dissected stelar root tissue. Oogonium walls enclosing the oospores are plainly visible in some cases. × 105. *B*, Mature oospore in oogonial envelope. Note the thick wall and the two spherical refractive bodies (one not in clear focus) at left edge of spore. × 750. *C*, 24-day-old culture on lima-bean agar. Approximately × 1/2. *D*, Mature and discharged sporangia from irrigated lima-bean-agar culture. Note proliferous type of branching in sporangiophore at top center. × 20. *E*, Sporangia on a diseased strawberry root tip. Upper right sporangiophore is sympodially branched. × 20.

from the disease as long as the soil temperature continues too high for the fungus to grow. This partial recovery is unavailing, however, as the fungus again becomes active with the advent of colder weather in the fall and the plant is attacked anew and more severely than before.

Positive diagnosis of the disease becomes much more difficult after the discolored-stele symptom is obliterated in early summer and can thereafter be made with certainty only by means of microscopic examination of roots. Thus, the period during which diagnostic macroscopic symptoms are present is relatively brief, a fact that should be particularly noted by nursery inspectors. Fall-dug plants may have incipient infections that easily escape detection because of their limited distribution and development, while it is almost impossible to diagnose light infections of the red stele disease during the summer months.

The most dependable proof that the red stele disease is present in suspected plants is the microscopic demonstration of oospores of *Phytophthora fragariae* in diseased roots. Oospores are produced almost exclusively in stelar tissue and as a rule are most abundant in the parts of the roots that were young when invaded. By making a thorough examination, the authors have never failed to find oospores in some part of every root known to be infected by the red stele organism. The oospores may be readily identified by their relatively large size, thick walls, and above all by the ever-present loosely fitting irregular-shaped oogonial envelopes, which enclose the oospores and impart to them a golden-brown or brick-red color (fig. 2, *A* and *B*).

THE PATHOGEN

The method of isolation of the cultures used in the present investigations has been described elsewhere (*6*) and need not be repeated here. A comparison of the authors' isolates with *Phytophthora fragariae* proved beyond question that the two are identical. After a comparative study of the group of *Phytophthora* spp. to which *P. fragariae* is most nearly related, and in view of the present chaotic state of classification within the genus, the authors agree with Hickman (*11*) that the strawberry red stele fungus should receive specific ranking, at least for the time being.

CULTURAL CHARACTERS

In its ability to grow in artificial culture media, *Phytophthora fragariae* appears to be intermediate between the majority of *Phytophthora* spp., which grow readily on a wide variety of artificial media, and the smaller group containing *P. phaseoli* Thax., *P. thalictri* G. W. Wils. and J. J. Davis, and *P. infestans* (Mont.) DBy., which grow much more slowly and on a more limited variety of media. Oatmeal agar, steamed bean pods, pea decoction, and lima-bean agar have proved favorable media, while the fungus either grows poorly or cannot be maintained at all after successive transfers on most common laboratory media such as corn-meal, potato-dextrose, and various malt-extract agars. Hickman listed Quaker-oat and French-bean agars as the most favorable media and lima-bean agar as fair and stressed as a classification feature the fact that the fungus does not grow on malt-extract agar.

The rate of growth of *Phytophthora fragariae* (fig. 3) is significantly slower than that of the following closely related species: *P. cryptogea* Pethyb. and Laff. (P–404, from Tucker through G. F. Gravatt), *P. cambivora* (Petri) Buisman (C–7, from Gravatt), *P. cinnamomi*
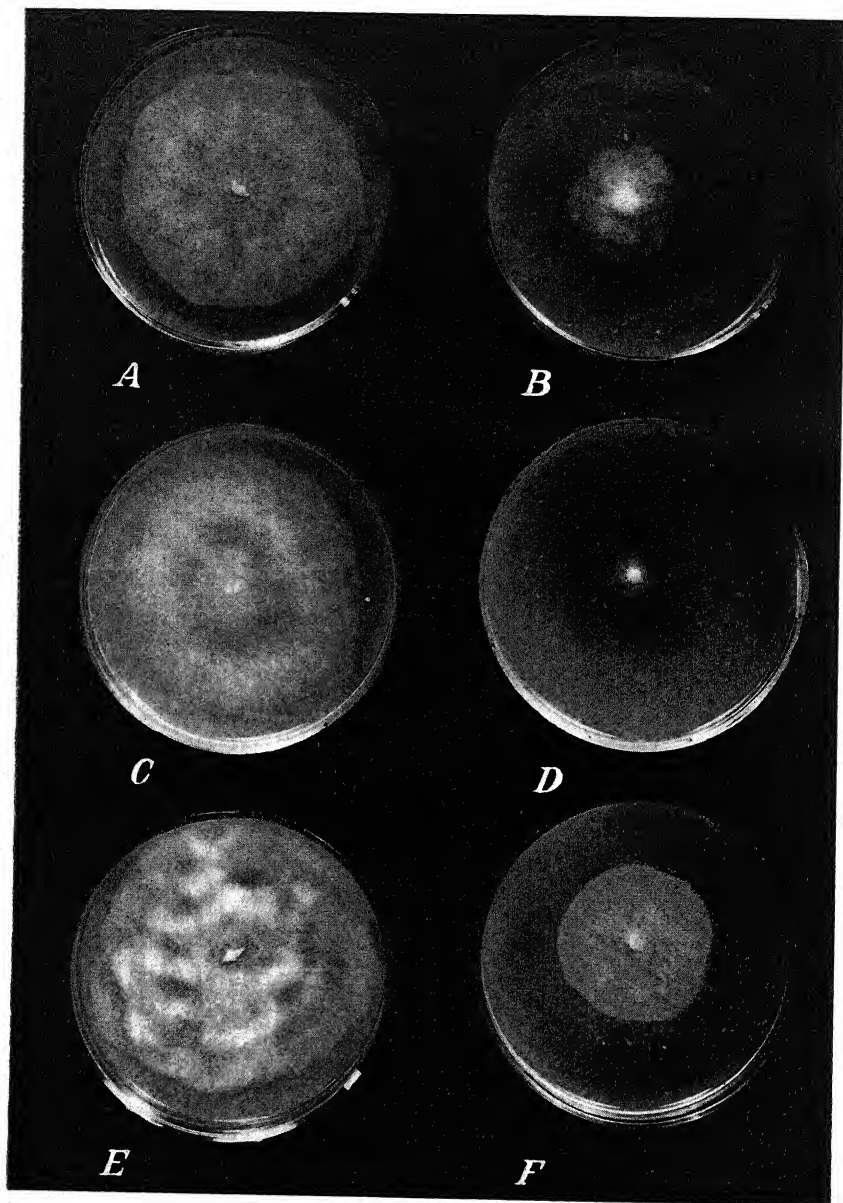
FIGURE 3.—*Phytophthora* cultures grown for 5 days at room temperature on lima-bean agar: *A, P. cryptogea* (P–404); *B, P. cambivora* (C–7); *C, P. cinnamomi* (B–5); *D, P. fragariae* (C–3); *E, P. drechsleri* (P–113); *F, P. megasperma* (P–340).

Rands (B–5, from Gravatt), *P. fragariae* (C–3, isolated from straw-berry root at Beltsville, Md.), *P. drechsleri* Tucker (P–113, from C. M. Tucker), and *P. megasperma* Drechs. (P–340, from Tucker). Hickman (*11*) gave the rate of growth of *P. fragariae* as 20 mm. in 5 days (agar and temperature not specified). The authors' measure-ments to determine the effects of temperature on rate of growth, given in table 1, were made with lima-bean agar as the substrate; the fungus, however, grows at a more rapid rate on oatmeal agar, as may be seen by comparing tables 1 and 2.

TABLE 1.—*Relation of growth of Phytophthora fragariae (culture C–3) on lima-bean agar to temperature*

Temperature (°C.)	Diameter [1] of colony	
	After 7 days	After 14 days
	Mm.	*Mm.*
4	0	Trace
10	13	22
14	18	33
18	23	36
22	25	38
25	16	16
30	0	0

[1] Average of measurements of 3 colonies at each temperature, expressed as averages between longest and shortest diameters when colonies were irregular in shape, the usual condition.

TABLE 2.—*Relation of growth of Phytophthora fragariae (culture C–3) at 22° C. to initial pH of oatmeal agar*

Initial pH	Diameter [1] of colony	
	After 7 days	After 14 days
	Mm.	*Mm.*
4.0	3	50
4.6	30	70
6.0	34	78
6.2	39	81
6.8	40	82
7.0	43	85
7.2	38	76
7.6	37	65
7.8	0	Trace
8.0	0	0

[1] Each value represents the average of measurements of 3 colonies, as in table 1.

While it is, of course, unwise to attribute too much importance to the rate of growth on artificial media, nevertheless when collections of different species of *Phytophthora* are grown side by side one cannot fail to be impressed by the conspicuously slow growth rate of *P. fragariae*, and it is noteworthy that this slow rate is consistent in all isolations of the fungus so far made.

Table 1 also brings out a relation that is considered to have even greater definitive classification value by most students of *Phytophthora*, namely, the minimum, optimum, and maximum temperature points for growth of the fungus. In a collection of more than 30 species of *Phytophthora*, Leonian (*15*) found only 6 that failed to make some growth at 31° C.: *P. cactorum* (Leb. and Cohn) Schroet. (1 of numer-

ous strains), *P. citricola* Saw., *P. hibernalis* Carne, *P. porri* Foister, *P. richardiae* Buisman, and *P. syringae* Kleb. Tucker (*22, table 24*) found that of a large collection of species and strains of *Phytophthora*, only *P. erythroseptica* Pethyb., *P. hibernalis* (which he subsequently merged with *P. syringae*), *P. infestans, P. richardiae*, and *P. syringae* failed to grow at 30°. Only the last 4 of these species had optimum growth temperatures below 25°. The optimum for the authors' strain of *P. fragariae* was evidently between 18° and 22°, as the rate of growth had declined sharply at 25°. Hickman (*11*) gave the following temperature relations for *P. fragariae*: Optimum growth at 20°, death at 30°, fair growth at 10°, and scarcely any at 4°. Thus, the relatively low values for both optimum and lethal maximum temperatures serve to distinguish *P. fragariae* from all but a few other species of *Phytophthora*.

The effect of hydrogen-ion concentration on growth of the fungus was determined by using as the substrate oatmeal agar adjusted to different initial pH values with hydrochloric acid and sodium hydroxide. Petri-dish cultures of the fungus were grown for 2 weeks at a constant temperature of 22° C. Table 2 gives the results. Under the conditions prevailing, *Phytophthora fragariae* grew at pH values ranging from the minimum one tested, pH 4.0, to 7.6; it grew most vigorously and rapidly between 6.0 and 7.2.

MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERS

SPORANGIA

Alcock (*1*) described the sporangia produced from diseased red core roots submerged in sterile water as follows: Nonpapillate, proliferous, usually inversely pyriform but variable in shape, variable in size, and averaging about 50μ by 30μ. Bain and Demaree (*6*) noted similar sporangia on diseased red stele roots and in irrigated pure culture of the fungus isolated from diseased roots.

Hickman (*11*) described the sporangia of *Phytophthora fragariae* as follows: Terminal on sporophores 10μ to 800μ long, nonpapillate, inversely pear-shaped, ovoid or ellipsoid, 32μ to 90μ by 22μ to 52μ, average 60μ by 38μ; both sympodial and proliferous types of branching of conidiophores present; sporangia produced in water, Petri solution, etc., but rarely on solid media.

As is to be expected, sporangia having the potentiality of reaching the unusually large size characteristic of *Phytophthora fragariae* vary considerably in both size and shape. In the authors' material, the sporangia emerging from diseased roots in tap water (fig. 2, *E*) measured 31μ to 70μ by 23μ to 38μ, and averaged 55μ by 33μ. In tap-water preparations of sporangia from mycelium grown on lima-bean agar (fig. 2, *D*), the corresponding dimensions were 39μ to 84μ by 28μ to 49μ and 65μ by 38μ, respectively. Figures 2, *D* and *E*, and 4, *A*, show some of the variations in size and shape. Malformed and dwarfed sporangia could be induced almost at will by growing the fungus in water from certain sources or by manipulating the temperature. Sporangia were never entirely normal in distilled water and often not in tap water, in which various amounts of chlorine and other solutes probably affected sporangial production. Water from melted snow proved excellent for inducing production of sporangia and zoospores. By using suitable water, the authors obtained zoospores in quantities

sufficient to inoculate several hundred potted strawberry plants without having to resort to the use of Petri solution or other similar methods for stimulating sporangial formation.

The effect of temperature on sporangial production was determined in routine preparations held continuously at various temperatures. The method of preparation suggested by Charles Drechsler, of this Division, was as follows: After the fungus had grown for 2 weeks or longer on thin layers of lima-bean agar in Petri dishes, the growth masses were cut into 5- by 10-mm. rectangular pieces, 20 or 25 pieces were transferred to a Petri dish, and sufficient water was added to barely cover the thinnest agar blocks in the dish. Sporangia developed most abundantly on sporangiophores growing into the water from the
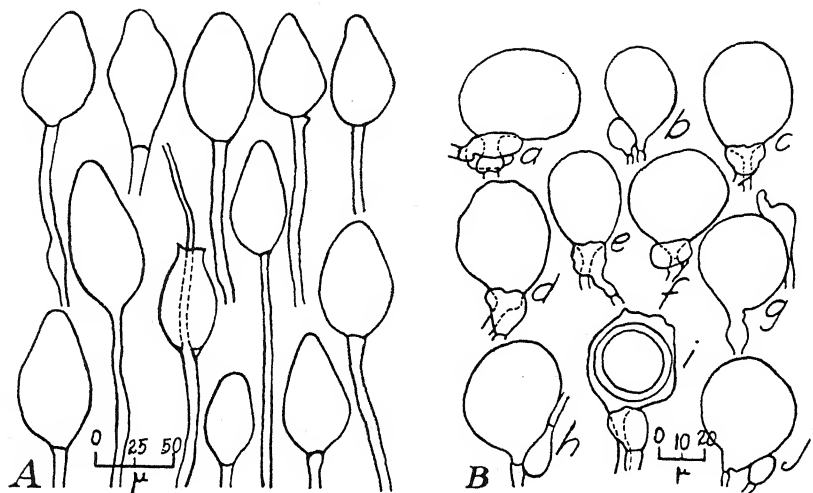
FIGURE 4.—*Phytophthora fragariae:* A, Sporangia, showing range in size and shape, $\times$ 200; B, a–j, oogonia and antheridia, $\times$ 300.

edges of the blocks (fig. 2, D), although sometimes they were produced copiously over the entire upper surface. Sporangial production was most vigorous and abundant at 14° C., slightly less so at 18°, fairly abundant at 10° and 22°, and occasional at 25°. At the last-mentioned temperature there was no zoospore discharge, and all sporangia eventually disintegrated completely. Not many sporangia discharged zoospores at 22°. The optimum temperature for sporangial and zoosporic production thus appears to be several degrees lower than the optimum for mycelial growth. At 22° and 25° sporangia began to appear 1 or 2 days after the preparations were set up, while at the lower temperatures 3 or 4 days, sometimes even longer periods, were required. If the water was changed daily at the favorable temperatures, sporangia continued to form for 2 weeks or longer. Some evidence was obtained that fluctuating temperatures may be more conducive to sporangial and zoosporic production than constant temperatures.

It remains controversial whether sporangial characters in *Phytophthora* spp. have much classification value, but most investigators

agree that the presence or absence of a papilla separates the species into two general groups. On this basis, *P. fragariae* would be grouped with the nonpapillate species *P. cambivora*, *P. cinnamomi* (Mehrlich (*16*) advocated merging *P. cinnamomi* with *P. cambivora*), *P. cryptogea*, *P. drechsleri*, *P. erythroseptica*, *P. hibernalis* (synonymous with *P. syringae* according to Tucker (*22*)), *P. megasperma*, *P. porri*, *P. richardiae*, and *P. syringae*.

Zoospores

As noted by Hickman (*11*), *Phytophthora fragariae* has zoospores typical of the genus; they are irregularly ellipsoidal, grooved on one side, and biciliate, round up on resting, are 12μ in diameter, occur generally 40 to 50 per sporangium, and germinate by germ tube, sometimes in the sporangium. To this description the authors can add little. Zoospores were usually about 10μ in diameter after coming to rest.

To determine the duration of the free-swimming period of zoospores, the authors transferred a concentrated suspension that had been discharging from sporangia since 11 a. m. to a Petri dish at noon and held it at $10°$ to $15°$ C. for the duration of the test. At 1:30 p. m. the spores were still swimming freely. At 2 p. m. most were swimming, some had encysted, and a few had already germinated. At 3:30 p. m. there was a conspicuous reduction in numbers and activity of swimming zoospores, but the percentage of germination in encysted spores was not high. The last observation was made at 4:15 p. m., when only an occasional spore could be found swimming. Germination was still poor.

Although the authors did not succeed in determining temperature limits for zoospore germination, they found germinated spores most often at $10°$ and $14°$ C. Zoospores are evidently exceedingly sensitive to the temperature as well as to the composition of the liquid in which they are released.

A final observation appears worth recording. On one occasion when a large number of zoospores was desired, a heavy crop of sporangia developed in the daytime and appeared likely to mature and discharge spores during the night. In an attempt to arrest the discharge, the whole lot, consisting of some 50 Petri-dish preparations, was poured into a 2-liter beaker, which was then filled with water and placed outside the laboratory window at near-freezing temperature. No swarm spores had been discharged at the end of the working day. When the beaker was examined the following morning, a thin film of ice covered the surface of the water, but most of the sporangia had nevertheless succeeded in discharging zoospores, vast numbers of which were actively swimming in the icy water.

The Sexual Stage

It is almost impossible to obtain satisfactory material for precise morphological study of the sexual stage of *Phytophthora fragariae*. Hickman (*11*) stated that in his material the fungus occasionally produced a few scattered oospores in oatmeal-agar cultures, but that such spores were too closely associated with fragments of oat kernels or hulls to be of much value for morphological study; he made no mention of oospores in or on any other medium. In the authors' isolates the

sexual stage failed to develop in any of the artificial media used, except in old oatmeal-agar cultures and in steamed string beans, where a few red oogoniumlike bodies of indeterminate structure could be found. Mating different isolates of the fungus yielded no evidence of heterothallism; on the contrary, the unmated strains invariably produced oospores when inoculated separately into their natural medium, the roots of growing strawberry plants. Inability of *Phytophthora* cultures to produce the sexual stage in artificial media is of course considered to be a classification character of some importance, but unfortunately it limits the study of this stage of the fungus to material occurring naturally in the host plant.

Sexual organs of the red stele root disease fungus as a rule develop only in central cylinders of infected roots. This region of compact cellular tissue is stained so deeply as a result of fungus penetration that minute details of young reproductive structures cannot be made out in fresh material. Prepared slides proved equally unsatisfactory for studying the process of sexual reproduction.

The best results were obtained in whole mounts of steles dissected free from the cortex and decolorized with a weak bleaching-powder solution, then stained very lightly with gentian violet, cotton blue, or some similar stain, and cleared in dioxane and mounted in water or cleared and mounted in lactophenol solution. Both treatments disorganized the oily contents of the reproductive organs but left the cell walls intact in their natural positions. The relation between antheridia and oogonia could be most reliably made out in the early stages of fertilization, before oospores were delimited within the oogonia. In figure 4, *B*, only 1 oogonium (*i*) out of the 10 illustrated contains a mature oospore.

Study of material prepared as described raised a question whether the antheridia are predominantly amphigynous as first stated by Alcock (*1*) and later by Bain and Demaree (*6*) and Hickman (*11*). It is true that antheridia are usually closely attached to the bases of oogonia, but after prolonged search the authors did not find a single instance in which they could be sure that the oogonial stalk actually penetrated the antheridium. On the contrary, it is not difficult to find favorably oriented material in which the association is unmistakably paragynous (fig. 4, *B*, *b*, *g*, *h*, *j*). Two cases that might possibly have been amphigynous are illustrated in figure 4, *B*, *a* and *f*. In the other illustrations (fig. 4, *B*, *c*, *d*, *e*, *i*), which are typical of most cases seen in roots, the antheridia were situated entirely above the oogonial stalks, although at first sight they appeared to be amphigynous. Alcock, Howells, and Foister (*3*) eventually concluded that the two types of fertilization take place in about equal proportions. Satisfactory study of minute details of fertilization must await the discovery of a medium other than living roots in which sexual reproduction will take place freely.

Grosser reproductive structures are easily made out. Hickman's (*11*) description may be summed up as follows: Oogonia terminal or lateral, variable in shape, 28μ to 46μ (average 39μ) in diameter, wall thick, smooth, golden brown with age; antheridia usually terminal, amphigynous or less commonly paragynous, 16μ to 30μ by 12μ to 22μ (average 22μ by 16μ); oospores spherical, ellipsoidal or more irregular, diameter of spherical spores 24μ to 44μ (average 33μ); oospore wall 3μ

thick; oospore containing a single large central oil globule. The authors consider that this central area is a vacuole, not an oil globule.

The oogonia tend toward ellipsoidal to subglobose shapes in the earlier stages, but they accommodate themselves somewhat to the limitations of the crowded region in which they develop (figs. 2, *A* and *B*, and 4, *B*). After the oospores are differentiated, the oogonia lose their turgidity, but the partially collapsed walls persist as tough, loosely fitting envelopes, which in time become dark red to golden brown. In the authors' material 125 oogonia measured 29μ to 75μ by 26μ to 52μ and averaged 55μ by 39μ.

The spore walls are perfectly smooth and the spores themselves are colorless, as may be demonstrated by dissecting off the colored oogonial envelope; in mature spores there are typically 2 lenticular "refractive bodies," 3.5μ to 6.5μ in maximum diameter, embedded in the peripheral cytoplasmic layer (fig. 2, *B*). In the authors' material 200 mature spherical oospores ranged from 20μ to 46μ in diameter and had a mean of 33.5μ. Elliptical spores usually occurred in the ratio of 1 to 4, and the maximum deviation from spherical shape was a 20-percent difference in lengths of major and minor axes.

The sexual characters most commonly considered to have taxonomic importance in *Phytophthora* spp. are type of antheridium, size of oospore, and ability of the fungus to produce the sexual stage in artificial media, either directly or after certain manipulations. As knowledge concerning the behavior of *Phytophthora* spp. gradually accumulates, these characters are frequently found to vary more within described species than between species, resulting in much taxonomic confusion, and indeed leading Leonian (*15, p. 33*) to hold that there are probably not more than three true species in the genus.

In the characters just mentioned, the red stele fungus compares with its most nearly related species as follows: If antheridia should prove to be always predominantly paragynous, the fungus would belong with the group containing *Phytophthora syringae*, *P. hibernalis*, *P. porri*, and *P. megasperma*. These four species, like *P. fragariae*, have unusually low temperature-growth relations. (Tucker (*22*) reduced *P. hibernalis* to synonymy with *P. syringae;* the description of *P. porri* (*9*) was evidently not available at the time of his publication. Leonian (*15*) retained the first three species, but recognized their close relationship. The temperature relations of *P. megasperma* are given by Wager (*23, table 3*).) If, on the other hand, the red stele fungus eventually proves to be predominantly amphigynous, as it has usually been considered up to the present time (*1, 6, 11*), it would be grouped with *P. cambivora*, *P. cinnamomi*, *P. cryptogea*, *P. drechsleri*, *P. erythroseptica*, and *P. richardiae*.

Previous to the description of *P. fragariae*, only two species of *Phytophthora* having oospores with an average diameter greater than 30μ in the respective host plants had been described. They were *P. megasperma* (*8*) in the paragynous group, with oospores averaging 41μ in diameter, and *P. erythroseptica* in the amphigynous group, whose oospores have been variously described with 31μ to 37μ average diameters. In these two species, spore size has been considered a valid diagnostic character. If this precedent is followed, the oospore size of *P. fragariae* (about 33μ average diameter) must be considered sufficiently distinctive to separate it from all described species of

Phytophthora except *P. erythroseptica*.   Indeed, Tucker (*22, p. 180*) suggested that these two species might be identical merely on the basis of Alcock's original description of the red core root fungus from its host plant (*1*).   However, even if *P. fragariae* eventually proves to belong in the amphigynous group with *P. erythroseptica*, certain cultural and host relations recounted later appear to warrant separating the two species.

According to Tucker (*22*), *Phytophthora erythroseptica* produces oospores rather abundantly in old oatmeal-agar cultures; Rosenbaum (*19, table 1*) stated that oospores were produced on a number of media; Leonian (*15, table 4*) found that oogonia developed when mycelial mats grown in pea broth were transferred to distilled water.   In its ability thus to produce oospores, *P. erythroseptica* differs from *P. fragariae*, for, as has been pointed out previously, the latter consistently fails to develop the sexual stage on artificial media under these as well as all other conditions tried, except for the sparse production of doubtfully functional oosporelike structures in old oatmeal-agar cultures and on steamed string beans.   The temperature-growth relations of the two species are also significantly different.

The authors observed oospore germination in only 1 instance.   In February 1940, 50 oogonia containing mature oospores were dissected from diseased roots and after being transferred to hanging-drop water cultures in 5 Van Tieghem cells were kept in a refrigerator at a temperature of 10° C.   No germination was noted during the next 3 months.   The slides were then left undisturbed in the refrigerator until early August.   At that time only 1 slide had not dried out.   In it 1 spore had germinated sometime previously, giving rise to a long-stalked sporangium which had discharged its contents and had been followed by 1 additional proliferous sporangium which likewise appeared to have discharged zoospores in a normal manner.   Several recently formed sporangia were found at the same time in some water-agar Petri-dish preparations of similar age on which entire steles instead of isolated oospores had been placed.   These sporangia probably came from germinated oospores, but connections between sporangia and oospores could not be traced with certainty.

The authors have made continuous but wholly unsuccessful efforts to follow the entire course of development of oospores in strawberry roots.   The spores obviously require a resting period before germinating, as roots infected during the fall-winter-spring period contain a high proportion of normal-appearing oospores throughout the succeeding summer.   Under field and greenhouse conditions, such roots have largely rotted away by the following spring and it is rare to find normal-appearing spores in the few that remain.   During the fall season, after the summer rest period, the proportion of apparently normal spores in these old roots decreases rapidly, presumably in large measure as the result of germination.

Oospore behavior is unquestionably of extreme importance to the incidence and perpetuation of the disease.   Throughout the greater part of the United States summer temperatures are undoubtedly high enough to kill out the mycelial stage of the fungus, and the oospores consequently must serve to oversummer rather than to overwinter the disease.   The maximum temperature that oospores are able to withstand is not known and cannot be determined until a

reliable method of germinating the spores is discovered; indirect evidence on this point, however, is presented below under the account of a hot-water-treatment experiment. It is also noteworthy that the disease invariably recurs in potted plants held over summer in the greenhouse at the Plant Industry Station, Beltsville, Md., where for weeks at a time the daytime temperatures to which the pots are subjected vary from 90° to well over 100° F. Judging from the comparatively narrow range of artificial media on which the fungus thrives, from its inability to produce oospores in the absence of its host plant, from the sensitivity of its mycelium to high temperatures, and from its limited host range (p. 502), it appears unlikely that the fungus can maintain itself in the soil if strawberry plants are not available at the critical period when oospores are germinating. Thus longevity of oospores may well determine the length of time the disease can persist in soils kept free from strawberry plants.

INOCULATION EXPERIMENTS

PATHOGENICITY OF PHYTOPHTHORA FRAGARIAE TO CULTIVATED STRAWBERRY

Since the authors have already presented evidence establishing the pathogenicity of their strains of *Phytophthora fragariae* (*6*), it is unnecessary to describe such experiments in detail here. Figures 5 and 6 illustrate the results obtained in an early experiment of this sort,
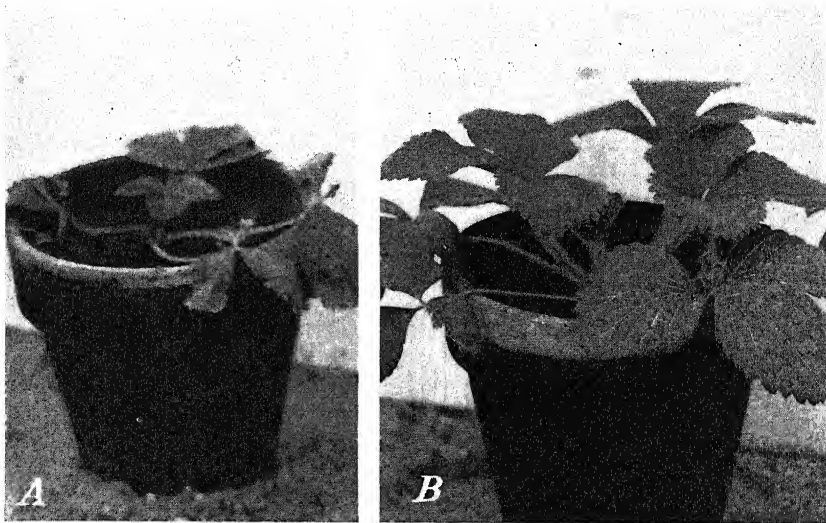
FIGURE 5.—*A*, Strawberry plant 3 weeks after inoculation with *Phytophthora fragariae*. *B*, Noninoculated check plant.

in which zoospore suspensions in sterile water were poured over the roots of young strawberry plants of the variety Blakemore growing in pots in autoclaved soil. After inoculation these plants were held for 3 weeks in fairly wet soil in a greenhouse at a temperature of 50° to 60° F. As a consequence of the mass inoculation, all main roots on these small plants became infected simultaneously and the wilting
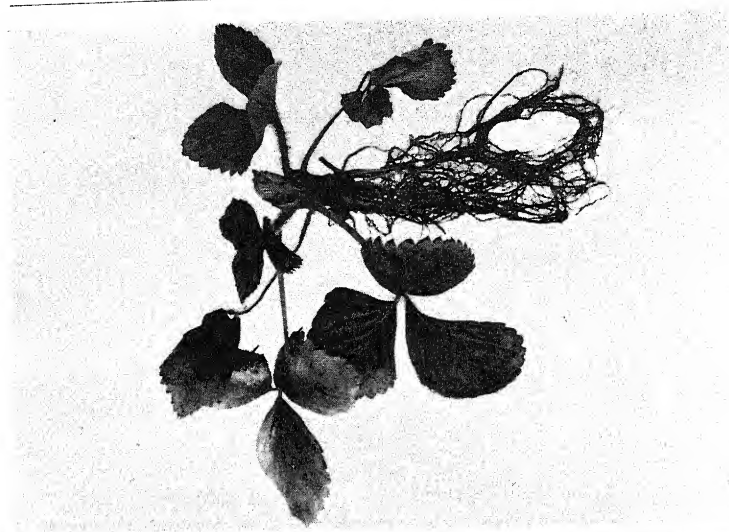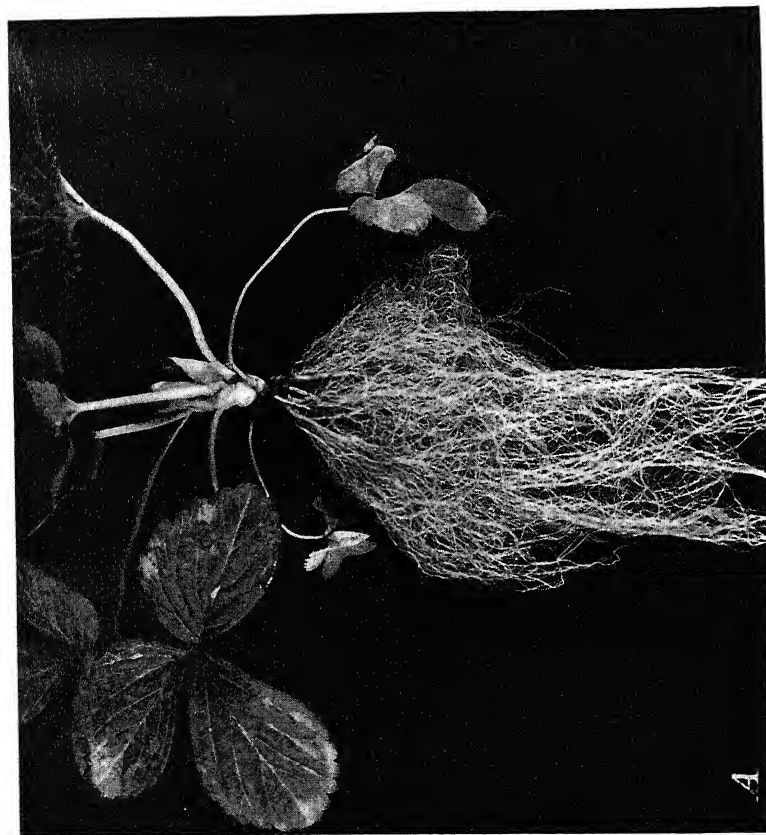
FIGURE 6.—Plants from the same lot as those shown in figure 5: *A*, Noninoculated plant; *B*, inoculated plant, showing destruction of root system by the pathogen.

stage was reached in the short time of 3 weeks. *P. fragariae* was reisolated from the roots of the inoculated plants.

The isolate of *Phytophthora fragariae* used most frequently (C–3, isolated in November 1937) has been passed through and reisolated from strawberry roots 4 years in succession. When subcultures freshly recovered from strawberry roots were compared with older isolates, it was found that in culture the fungus gradually lost some of its capacity for producing sporangia but that the zoospores produced retained their pathogenicity unimpaired.

HOST RANGE OF PHYTOPHTHORA FRAGARIAE

SPECIES OTHER THAN FRAGARIA

Certain selected plants unrelated to *Fragaria* were inoculated to obtain information on the host range of *Phytophthora fragariae* and to compare it taxonomically with other *Phytophthora* spp. The method of inoculating roots of potted plants was to place blocks of mycelial tissue from Petri-dish cultures directly on the roots after the plant was removed from its pot, or, much more frequently, to pour a suspension of swimming zoospores over the roots; the plant was then returned to the pot and kept fairly wet in a cool greenhouse (55° to 65° F.). When parts other than roots were being inoculated, mycelium in masses sufficiently large to assure an abundance of viable inoculum was placed in incisions that were then either sealed with paraffin or wrapped with moist sterilized cotton and enclosed in Parafilm. Each test was usually made in triplicate and in some cases the same experiment was repeated several times. Because of the slow growth rate of the fungus, at least 2 weeks was allowed to elapse before final records of the results were taken.

Phytophthora fragariae failed to infect the following:

Potato tubers.—*P. erythroseptica* and *P. drechsleri* were originally described from this host; and, according to Tucker (22), *P. cinnamomi* and *P. cryptogea* are also pathogenic to the tubers.

Calla lily (roots of potted plants).—*P. richardiae* was described from this host and is said to be virulently pathogenic to it.

Leek leaves and roots.—The native host of *P. porri* (9).

Tomato seedlings and green fruits.—*P. cryptogea* was originally described from tomato stems and roots. Tucker (22) listed *P. cryptogea* and *P. drechsleri* as virulently pathogenic to tomato seedlings and *P. cryptogea* and *P. erythroseptica*, to green fruits; he listed *P. drechsleri* and one strain of *P. cinnamomi* as weakly pathogenic to the fruits.

Apple fruits.—Mycelium of *P. fragariae* grew a short distance into the tissues of one ripe Grimes Golden apple without causing rot, but it failed to grow at all in several others. Tucker's group virulently pathogenic to apple contains *P. cinnamomi*, *P. hibernalis*, and *P. syringae* and the weakly pathogenic group *P. cambivora*, *P. cryptogea*, *P. erythroseptica*, and *P. drechsleri* (22).

Eggplant fruits.—Tucker's virulently pathogenic group contains *P. cryptogea* and *P. drechsleri* (22).

Beet roots, green lima-bean pods, bean stems and roots, roots of *Potentilla monspeliensis* L., pansy roots, carrot roots.

Thus, *Phytophthora fragariae* has not infected the living tissues of any plant species so far tested outside of the genus *Fragaria*. Among the plants proved nonsusceptible there is at least one which is susceptible to each previously described nonpapillate species of *Phytophthora* except *P. megasperma*. The authors have not inoculated hollyhock, the native host of *P. megasperma*, with *P. fragariae*.

SPECIES AND VARIETIES OF FRAGARIA

The following species of *Fragaria* proved highly susceptible when inoculated with *Phytophthora fragariae*: *F. moschata* Duch., *F. virginiana* Duch., two strains of *F. vesca* L., and two of three strains of *F. chiloensis* (L.) Duch. The third strain of *F. chiloensis* did not become infected.

Some commercial strawberry varieties are known to be highly resistant to the red stele root disease (*14, 18, 20*). Of these, only the Aberdeen has so far been tested for resistance by direct zoospore inoculation of potted plants. Plants of this variety have never become infected despite repeated efforts to transmit the disease to them, although Anderson and Colby [13] have recently reported finding it slightly infected in the field in Illinois. Four resistant varieties developed in Scotland (*18*) and brought to this country for breeding purposes under P. I. [14] Nos. 127389 to 127392 have been made available through the courtesy of H. W. Anderson, of the University of Illinois. These, likewise, have proved wholly resistant when inoculated heavily with zoospores. The pot-inoculation technique is being used to supplement field tests in determining red stele resistance of hybrid seedlings now being developed in the strawberry breeding project conducted jointly by George M. Darrow, of this Division, and W. F. Jeffers, of the University of Maryland (*13, 14*).

PATHOGENICITY OF RELATED SPECIES OF PHYTOPHTHORA TO STRAWBERRY ROOTS

Groups of three potted Blakemore plants were repeatedly inoculated with mycelium, and with zoospores when available, of the nonpapillate species *Phytophthora cinnamomi*, *P. cryptogea*, *P. drechsleri*, *P. erythroseptica*, *P. fragariae*, and *P. megasperma*. The three plants inoculated with *P. fragariae* became infected and developed characteristic red stele disease symptoms. The remaining fungi without exception failed to infect roots of the plants on which they were placed. Noninoculated check plants likwise remained disease-free.

Cultures of the remaining nonpapillate species of *Phytophthora* either were not available or for other reasons were not tested against strawberries.

EXPERIMENTAL AND PRACTICAL CONTROL

In common with many root troubles, the red stele root disease in strawberries cannot be economically controlled by any known direct treatment of infected soil or diseased plants, but the problem of control must be approached indirectly by such means as growing resistant varieties, rotation of crops, fallowing infected fields as long as may be necessary to rid them of the fungus, and regulating the distribution of planting stock by individuals and more particularly by nurseries. Development of resistant strains by breeding appears to offer the most promising prospect of eventual commercial control of the disease. Various groups of plant breeders both here and abroad are at present engaged in the endeavor (*5, 7, 12, 13, 14, 18*).

It is not definitely known how long *Phytophthora fragariae* is able to persist in infected soil kept free from its only known host plant.

[13] ANDERSON, H. W. and COLBY, A. S. RED STELE OF STRAWBERRY ON PATHFINDER AND ABERDEEN. U S. Bur. Plant Indus. Plant Dis. Rptr. 26: 291–292. 1942.
[14] "P. I." refers to the accession numbers of the Division of Plant Exploration and Introduction.

Alcock and Howells (*2*) made some observations which indicated that the survival period might be 8 years or longer in Scotland. Some theoretical considerations discussed previously, based on temperature (p. 493) and nutritional reactions of the fungus, make it appear improbable that the fungus can survive for such long periods in most regions of the United States. A small test, started in May 1939, designed to furnish more definite data about the longevity of the fungus in infested soil, has already given some information. In that year strawberry plants well infected with *Phytophthora* incorporated with soil were placed in a shallow excavation in a field adequately isolated from growing strawberries. In March of the following year a sample of the soil was withdrawn, potted, and planted to 10 disease-free strawberry plants. In November of the same year, or 18 months after the test was started, another sample of soil was taken in which 20 plants were set. Both lots of plants were examined in April 1941. All 30 plants were badly infected with the red stele root fungus. In the autumn of 1941, or after a lapse of 2½ years, a third sample was taken in which 20 nondiseased plants were set. In this lot 1 plant only showed a trace of the disease the following spring (1942). None of the plants used as controls with the 3 lots developed the disease.

The authors are constantly on the lookout for some safe treatment of dug nursery plants that will kill the fungus in the root tissues of lightly infected plants which may have escaped detection during nursery inspection. Fungicidal treatments of this nature are carried out in cooperation with M. C. Goldsworthy, of this Division. So far no effective treatment has been found.

That hot-water treatments are likewise ineffective was proved experimentally by immersing badly diseased plants at different temperatures for different lengths of time and then potting an untreated disease-free plant with each treated plant in autoclaved soil. The experiment was started in the spring and the plants held under conditions favorable for both plant growth and fungus development until the following spring. Temperatures of 90°, 100°, 110°, 115°, and 118° F. were used, and time of immersion was varied from one-fourth hour to 5 hours. In all, there were 25 different temperature-duration series. Each series was replicated 10 times. Of the treated plants, 38 percent died, presumably as a result of the treatment. The roots of the dead plants were left undisturbed in the pots with the companion plants. The criterion of the effect of the treatments was the absence of the root fungus in the roots of the control plants at the termination of the test. The results of the test indicated that the pathogen was slightly more tolerant to the treatments than the plants. For evidence to support this conclusion, 2 examples may be cited. In plot 16, held at 110° for 2 hours, 8 of the 10 treated plants died, but the fungus spread to 5 of the control plants. In plot 18, held at 110° for 4 hours, all treated plants died, but 1 of the companion plants contracted the disease. At least 1 control plant of the 10 in each of the 25 temperature-duration series contracted the disease.

The red stele disease has so much potentiality for harm and is so difficult to cope with when once introduced that it is impossible to place too much emphasis on the importance of preventing its introduction into noninfested strawberry regions. With the present wide

distribution of the disease in the United States, this can be accomplished best by vigorous educational programs in conjunction with efficient inspection and certification systems applied to all nurseries and individuals distributing strawberry plants.

SUMMARY

The fungus causing the strawberry root disease known in the United States as black stele, red stele, or brown stele is shown to be identical with *Phytophthora fragariae* Hickman, which causes a similar strawberry root disease in Scotland and England known as Lanarkshire disease or red core root.

In this country the disease has been found in the following States: Arkansas, California, Connecticut, Delaware, Illinois, Indiana, Kentucky, Maryland, Massachusetts, Michigan, New Jersey, New York, Ohio, Oregon, Pennsylvania, Tennessee, Virginia, Washington, and Wisconsin.

Morphological and physiological studies of the fungus associated with the disease in the United States are described in detail.

Inoculation of roots, leaves, or fruits of plants other than strawberry known to be hosts to other *Phytophthora* spp. indicated *Fragaria* to be the only plant genus susceptible to infection by *P. fragariae*, and inoculations within the genus *Fragaria* showed that species and horticultural varieties of the cultivated strawberry vary in susceptibility to attack by the fungus.

The only practical methods of controlling the disease known at the present time consist in growing resistant varieties, keeping fields free of strawberries for at least 3 or 4 years after the soil has become contaminated, and preventing introduction of the disease into noncontaminated soils.

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THE INHERITANCE OF CERTAIN MUTANT CHARACTERS IN VENTURIA INAEQUALIS [1]

By J. R. SHAY, *research assistant*, and G. W. KEITT, *professor of plant pathology, Wisconsin Agricultural Experiment Station* [2]

INTRODUCTION

Certain nonpathogenic Ascomycetes, such as *Neurospora sitophila* Shear and Dodge and *Neurospora crassa* Shear and Dodge, have been shown to offer exceptionally favorable material for genetic studies. The work of Dodge and his associates (*2, 3, 4, 5, 19*), [3] Lindegren and his coworkers (*10, 11, 12, 13, 14, 15*), and others has greatly advanced knowledge of the mechanism of inheritance in this group of fungi. Keitt and his coworkers (*1, 6, 7, 8, 9*) have shown that the apple scab pathogen, *Venturia inaequalis* (Cke.) Wint., is very similar to the eight-spored species of *Neurospora* in adaptations for genetic work and affords favorable material for studies of inheritance of pathogenicity. These investigators have found only two types of pathogenic reaction, lesion and fleck, incited by monoascosporic lines freshly isolated from perithecia occurring in nature. They report that in all cases satisfactorily analyzed segregation of the factors for pathogenicity has occurred in 1:1 ratio, indicating that in a given isolate pathogenicity to a given variety is governed either by a single gene or by closely linked genes. They have also studied the cultural mutant characters *tan* and *nonconidial* and shown that the gene for either of these characters may suppress the expression of pathogenicity.

The present paper reports studies of inheritance of selected cultural mutant characters and sex reaction. Special attention was given to linkage relationships and to the effects of certain mutant genes on the expression of pathogenicity. A brief preliminary report of this work has been published (*17*). The background of the work and pertinent literature have been discussed in earlier papers (*6, 8*).

MATERIALS AND METHODS

The mutants dealt with in this study arose in Petri dish cultures of monoascosporic lines of *Venturia inaequalis* studied by Keitt and Langford (*6*). All except *small* appeared as conspicuous sectors that grew more rapidly than the lines from which they arose. All have been described in more or less detail by Keitt and Langford (*6*) or Keitt, Langford, and Shay (*8*).

White (W).—This mutant arose as a sector (C 5 sector of Keitt and Langford) in isolate 5 of ascus C. It is pinkish white on malt agar (pl. 2, *A*) and produces conidia abundantly. In matings with *normal* it is highly fertile, producing abundant perithecia, the asci of

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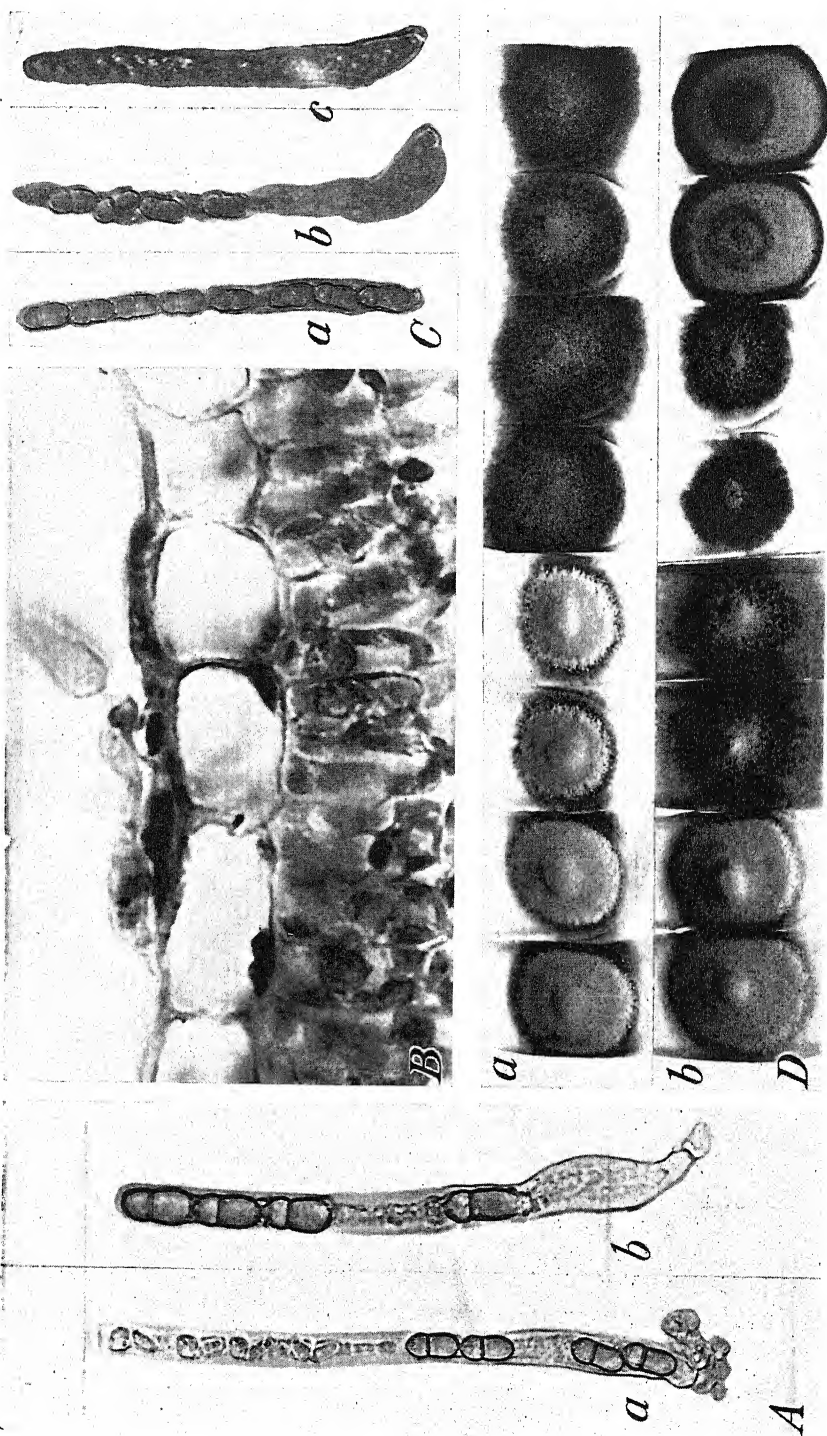
which contain four well-developed, *normal* ascospores and ·four that are partly or completely aborted (pl. 1, *A*). The partly aborted spores may be one- or two-celled and are usually misshapen and colorless. They are viable when sufficiently developed, and without exception the cultures grown from them have been *white*. In crosses of *white* × *normal*, white as well as typical dark ascocarps are produced, indicating that both the ascogonia and the antheridia produced in monoascosporic lines of this self-sterile hermaphroditic fungus are capable of functioning.

Tan (T).—This mutant arose as a sector (D 8 sector of Keitt and Langford) in isolate 8 of ascus D. It is characterized by tan color on malt agar and by producing conidia somewhat less abundantly than *normal* (pl. 1 *D, b*). Perithecia in crosses involving *tan* are produced sparsely, but the mutant has no aborting effect on the ascospores.

Nonconidial (Nc).—This mutant arose as a sector on the D 8 sector (*tan*) line of Keitt and Langford and was designated by them as sector from D 8 sector. It is characterized chiefly by the nearly complete suppression of conidial production and by its fine, compactly growing hyphae (pl. 1, *D, b*). On malt agar *nonconidial* cultures tend to be more grayish green than *normal* cultures.

Small (S). This mutant did not arise as a conspicuous sector but was discovered when an apparently *normal* sporulating isolate (one of eight *normal* lines taken 12 months previously from an ascus from an overwintered McIntosh leaf) was inoculated on McIntosh and failed to infect. Subsequent studies of the line established it as a mutant, but its use in this study was limited because it so nearly resembles *normal* in cultural characters, except for a somewhat smaller colony diameter, that it can be positively identified in the progeny only by the expensive and laborious process of testing pathogenicity.

In addition to the mutant characters, sex reaction (+ and −) was studied. The sex reactions of the lines are designated by the conventional +/− symbols. In previous work with *V. inaequalis* (6) sex reaction was tentatively designated by X and Y. The + sex reaction used throughout this paper is the X and the − is the Y

EXPLANATORY LEGEND FOR PLATE 1

A, Asci of *Venturia inaequalis* from a *white* × *normal* cross showing partial (*a*) and complete (*b*) abortion of ascopores carrying the *white* gene. *a*, × 429; *b*, × 531. *B*, The *small* mutant (line F 7) after 5½ days on McIntosh apple leaf, showing penetration mechanism and the restricted subcuticular development of the thallus. The outline of another conidium may be seen above the appressorium. × 600. *C, a*, eight-spored ascus typical of those from a *normal* × *normal* cross; *b*, four-spored ascus from a mating of a *normal* with a mutant, inducing complete ascospore abortion; *c*, ascus containing no spores, from a cross involving two compatible mutants each of which induces complete ascospore abortion. *a, b, c*, × 365. *D*, Sets of cultures from the ascospores of two asci from the *tan nonconidial* × *normal* mating, after 30 days in agar slants. The cultures are arranged in serial order with that arising from the No. 1 ascospore at the left. In *a*, it is shown that both *tan* and *nonconidial* segregated in the first meiotic division and were so distributed that four double mutants (*tan nonconidial*) and four *normal* cultures resulted. In *b*, it is seen that *tan* segregated in the first division and *nonconidial* in the second, giving the following distribution: 1, 2 *tan nonconidial*; 3, 4 *tan;* 5, 6 *normal;* 7, 8, *nonconidial*. Both *a* and *b* approximately natural size.
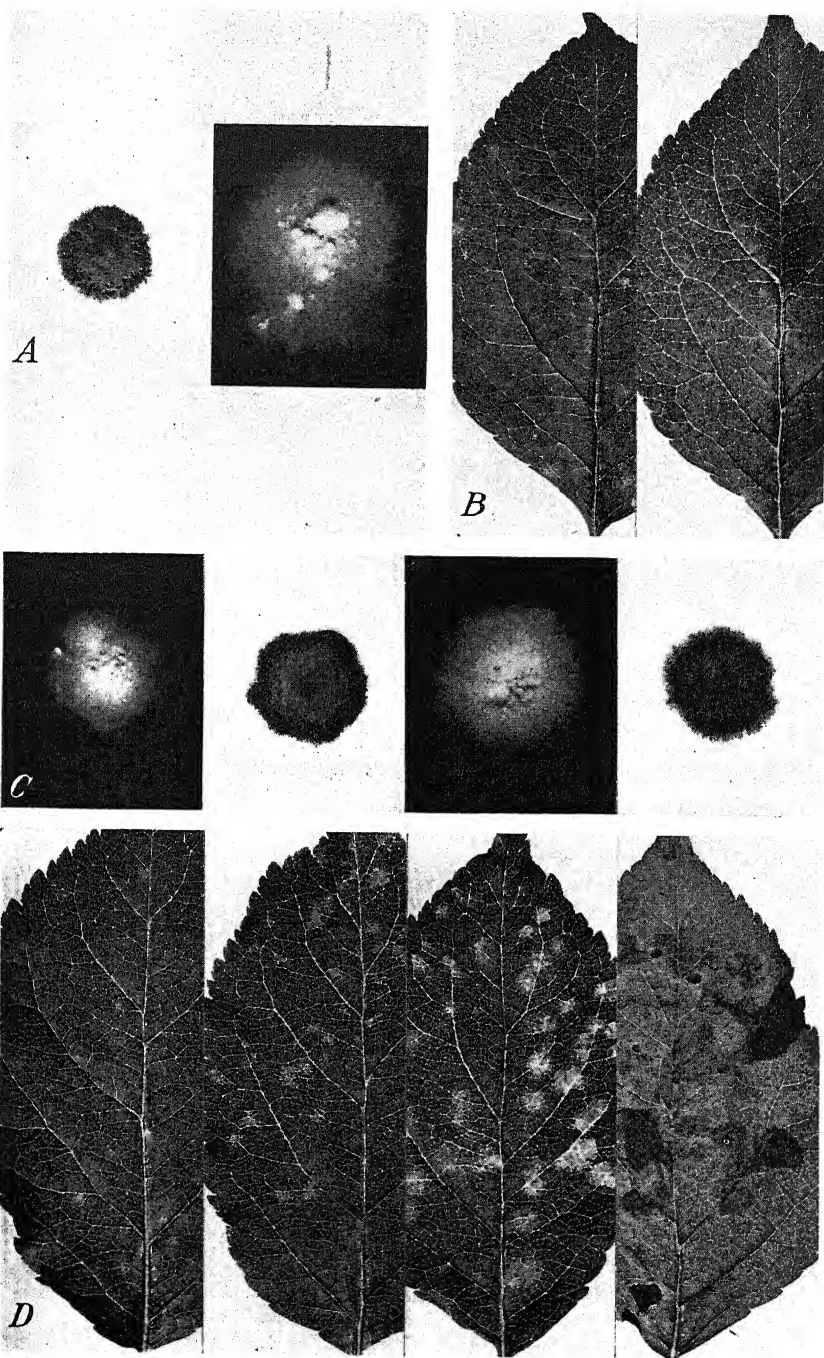
reaction reported by Keitt and Langford (*6*). Since none of the factors dealt with in this study are linked with sex reaction, there are no data from which conclusions as to the true nature of differentiation for sex reaction can be drawn. It is possible that future work will reveal that the $+/--$ factors herein treated as alleles are differentiated regions of a chromosome or that some other mechanism of differentiation for sex reaction exists. However, recent work in *Neurospora sitophila* (*18*), in which the gene *pink* is located distal to the sex factors, gives support to the allelomorphic $+/-$ designation.

Normal denotes the typical, dark-colored, sporulating, wild-type culture.

The mutant and *normal* lines of *V. inaequalis* used in this study were carried in stock culture on Trommer's malt extract (2.5 percent) agar (1.7 percent). They were transferred at 8-week intervals by the monoconidial method (except in the case of *nonconidial* cultures, in which hyphal tips were used) described by Keitt and Langford (*6*).

The lines were mated in vitro and held at 6° to 8° C. after being at 20° C. for the first 10 days after seeding. Trommer's malt extract (0.5 percent) agar (2.5 percent) with a decoction of dead McIntosh apple leaves (25 gm. leaves per liter of medium) was used to obtain the perfect stage as described by Keitt and Langford (*6*). Usually about 4 months elapsed before the asci contained ripe ascospores suitable for isolation. When the asci were mature, the ascospores were isolated in serial order by the glass-needle method described by Keitt and Langford (*6*). After germination each ascospore was transferred to a test-tube slant of Trommer's malt extract (2.5 percent) agar (1.7 percent) and incubated at 16° C. for 4 weeks or until sufficient growth had occurred to permit determination of the cultural characters under investigation (pl. 1, *D*).

The method developed by Lindegren (*10*) for locating genes in Ascomycetes with reference to their centromeres or spindle-fiber attachments was used. One-half the percentage of asci showing second-division segregation for a given gene pair is taken as the uncorrected distance in cross-over units of the gene from its centromere. Lindegren's method has been criticized by Wülker (*20*) and Zickler (*21*). A discussion of these criticisms appears to be unwarranted in the present paper.

The pathogenicity tests were performed on trees in the greenhouse. The method of growing the trees and the inoculation technique used have been described by Keitt and Langford (*6*) and Keitt, Langford, and Shay (*8*).

EXPLANATORY LEGEND FOR PLATE 2

A, Parent isolates, *Venturia inaequalis*, D 2 *normal* (left) and C 5 sector *white* (right); monoconidial cultures, 8 weeks old. *B*, Red Astrachan leaves inoculated, respectively, with the isolates in corresponding positions in *A*: Left, flecking without sporulation; right, no macroscopic infection. *C*, Isolates representing the four biotypes (two *white* and two *normal*) from an ascus (XI) obtained from mating the lines shown in *A*. Isolates 1, 2, 5, and 6 (left to right), numbered in the order of occurrence in the ascus of the ascospores from which the cultures were derived; monoconidial cultures, 8 weeks old. *D*, Red Astrachan leaves inoculated, respectively, with the isolates in corresponding positions in *C*; two on left show flecking with no sporulation; two on right show sporulating lesions, one white and the other typically dark. *A* and *C*×ca. ½; *B* and *D*×ca. ¾.

EXPERIMENTAL RESULTS
LOCATION OF GENES

The percentages of second-division segregation for *white, tan, nonconidial, small,* and the sex reaction factors are shown in table 1. To determine whether the *white* gene pair segregated in the first or the second division, it was not necessary to dissect the asci but only to examine them microscopically. The arrangement of the nonaborted and the aborted spores could be readily and accurately determined (pl. 1, *A*). *White* and *tan* are nearest the centromere, being, respectively, 6.0 and 10.3 cross-over units distant. *Nonconidial* is relatively distant at 19.6 units from the centromere. The number of asci studied for the location of *small* is not sufficient to determine the location accurately, but the indications are that the gene is at a considerable distance from the centromere. The factors for sex reaction are also relatively far out on the chromosome arm. None of the distances given are corrected for double cross-overs.

Consideration was given to the possibility that passing of the two middle nuclei in the four-nucleate ascus (*15, 18*) might occur in *Venturia inaequalis* and constitute a factor of error in these studies. Of the asci segregating for the *white* alleles, 88 percent showed segregation in the first division. If nuclear passing occurred frequently, there would be an excess of the alternate over the symmetric arrangement in those asci in which the arrangement of spores suggested second-division segregation. In a determination involving 169 asci showing second-division segregation of the *white* alleles, 80 showed the alternate (*w W w W* or *W w W w*) and 89 the symmetric (*w W W w* or *W w w W*) arrangement. Determinations in other crosses did not give evidence of any significant excess of the alternate arrangement in asci showing second-division segregation. Although the numbers of asci examined in these latter cases were not considered adequate, the data as a whole indicate that if nuclear passing occurs at the four-nucleate stage, it is not a factor of much importance in the studies reported in this paper.

TABLE 1.—*Summary of data on the distance from the centromere in cross-over units of several mutant genes and the sex reaction factors in Venturia inaequalis*

Character	Mating	Asci studied	Asci showing segregation in division—		Second division segregation	Distance of gene from centromere in cross-over units
			I	II		
		Number	*Number*	*Number*	*Percent*	*Number*
White	$W \times w$	1,804	1,588	216	12.0	6.0
Tan	$T \times t$	71	56	15	20.6	10.3
Tan	$T \times t$	138	110	28		
Nonconidial	$Nc \times nc$	138	84	54	39.1	19.6
Small	$S \times s$	8	4	4	50.0	25.0
Sex reaction	$+ \times -$	43	18	25	58.1	29.1

LINKAGE RELATIONSHIPS

The associations of *white, tan, nonconidial,* and the sex-reaction factors as determined by the proportion of new to parental combinations appearing in the progeny of crosses involving these genes are

TABLE 2.—*Linkage relations of white, tan, nonconidial, and the sex-reaction factors in Venturia inaequalis*

Mating	Asci studied	Pairs of ascospores [1]	Progeny combinations		New combinations	Conclusions
			Parental	New		
	Number	*Number*	*Number*	*Number*	*Percent*	
W+×w-	41	164	70	94	57.3	}Independent.
w+×W-	8	32	16	16	50.0	Do.
T+×t-		260	120	140	53.8	Do.
Nc+×nc-		258	126	132	51.2	Do.
Wt×wT	42	168	160	8	4.8	Linked.
TNc×tnc	138	552	288	264	47.8	Independent.

[1] The ascospores of each pair are genetically identical and constitute one of the 4 biotypes resulting from a meiosis.

shown in table 2. In most of the asci involving *white*, only four of the ascospores (*nonwhite*) were recovered, as all ascospores carrying *white* were partly or completely aborted and many were therefore nonviable. The genotype of the four ascospores bearing *white* could be accurately deduced from the four *nonwhite* ascospores recovered. For example, *tan* and *white* were found to be linked. Thus, an ascus from the *tan*×*white* cross in which the four nonaborted ascospores gave rise to two *normal* and two *tan* cultures would contain a crossover. The four aborted ascospores in such an ascus, had they been recovered, would have given rise to two *white* and two *tan white* cultures. Similarly, the sex reaction of nonrecovered aborted ascospores could be deduced when that of the recovered nonaborted ascospores was ascertained.

Matings involving mutants are frequently much less fruitful than those involving only *normal* lines, and this unfruitfulness increases in cases of multiple mutants. For this reason the use of more than two or three mutant characters in a mating is difficult, for very few or possibly no perithecia are likely to result. Negative results were encountered on many occasions when *tan* and *nonconidial* lines were mated with *normal* of opposite sex reaction to determine whether these two mutant genes were linked with the sex-reaction factors. The data for complete asci were not always obtained, but the parental and new combinations that were obtained from a given ascus are recorded in table 2 by pairs of ascospores. For example, in an ascus containing *tan*, *tan nonconidial*, *nonconidial*, and *normal*, all but *normal* may fail to produce perithecia when tested for sex reaction. When the sex reaction of the *normal* pair of ascospores is determined, one of the *tan* pairs and one of the *nonconidial* pairs are known to be of the opposite sex reaction. Two pairs of ascospores from this ascus can then be recorded.

Because of the large numbers of trees that would have been required to identify *small* in the progeny, this character was not used in the linkage studies.

The data in table 2 show independent assortment for all genes studied except *tan* and *white*. The latter appear to be rather strongly linked with a distance of 4.8 cross-over units separating them. These data, in conjunction with the data from second-division segregation of these two gene pairs, permit the construction of a two-point chromosome map (fig. 1). *Tan* appears to be located at a distance of
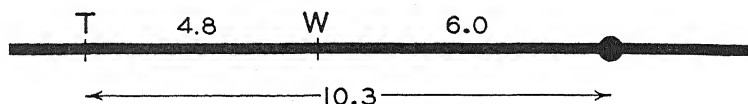
FIGURE 1.—Two-point map of the first chromosome in *Venturia inaequalis*.

10.8 units from the centromere and distal to *white* on what is here termed the first chromosome. The distance (10.3) of *tan* from the centromere as obtained from the frequency of second-division segregation is in good agreement with the sum (10.8) of the distance of *white* (6.0) as obtained from second-division segregation data (table 1) and the distance between *tan* and *white* (4.8) as determined by recombination (table 2). It can readily be seen that the data on recombination are from a source independent of these from second-division segregation.

PATHOGENICITY STUDIES OF *WHITE* AND *SMALL*

The original *white* mutant as reported by Keitt and Langford (*6*), and designated C 5 sector, was nonpathogenic to the Red Astrachan apple variety. This finding was confirmed in the present study. It is of interest, however, that the *white* progeny from a cross of *white*×*normal* are pathogenic. Results were obtained from but one ascus (designated ascus XI), of which the four biotypes are shown in plate 2. Three of the aborted *white* spores were viable, but the fourth (No. 8 in the ascus and paired with No. 5) failed to germinate. Considerable shifting of position occurred in the ascus, but this is not infrequent in asci containing aborted spores. The *normal* parent of the ascus (isolate 2 of ascus D of Keitt and Langford) incites the fleck reaction with Red Astrachan. The *normal* line from which *white* arose as a sector (isolate 5 of ascus C of Keitt and Langford) incites the sporulating lesion reaction with Red Astrachan. From the ascus of which the four biotypes are illustrated in plate 2, isolates 1, 2, 3, and 4 incite flecking on Red Astrachan; whereas 5, 6, and 7 incite sporulating lesions. Isolates 1, 4, and 5 are *white*. The No. 8 aborted (*white*) spore that failed to germinate would have incited sporulating lesions.

As stated earlier, the mutant *small* is nonpathogenic to all the varieties of apple on which it has been inoculated. Since the line produces abundant conidia in culture it became of interest to know at what point the mutant failed to establish pathogenicity. A histological study of the original mutant *small* on leaves of the McIntosh apple variety was undertaken, employing the methods used by Nusbaum and Keitt (*16*) for inoculating, killing, fixing, dehydrating, and embedding the tissue. Collections of inoculated leaf material were made at intervals up to 5½ days after inoculation. Heidenhain's iron-alum haematoxylin stain was used. Plate 1, *B*, shows the mutant *small* at 5½ days after inoculation. The fungus has penetrated in the typical way, but its subcuticular development has been sharply arrested, and is confined to a diameter of approximately three times the length of the conidium. This is in sharp contrast to the development of *normal* lines that incite the sporulating-lesion type of reaction (*16*). It is not known how much greater development of the incipient lesion would have occurred, but the readings of the results of inoculation studies using the progeny of *small*×*normal* were taken some 3 weeks subsequent to inoculation, and no macroscopic evidence of infection could be discerned.

DISCUSSION

The present work was undertaken as a step in exploring the possibility that genetic studies of cultural mutants of *Venturia inaequalis* may furnish information or materials of value in studies of inheritance of pathogenicity. The mechanism of inheritance in *V. inaequalis* appears to be essentially the same as that of the more extensively studied *N. crassa*. However, as has been pointed out in earlier papers, minor changes of position of nuclei or spores are not infrequently encountered in *V. inaequalis*. Usually the line of nuclear descent can be clearly deduced. In some cases, however, this is not possible. While these irregularities might preclude the use of this fungus for detailed studies of certain highly specialized genetic problems, such as chromatid interference in crossing over, they have not occasioned an important source of error for the studies reported in this paper.

The occurrence of the degenerate ascospore abortion character (pl. 1, *A*, *C*) is frequent among cultural mutants of *V. inaequalis*. Keitt and Langford (7) state: "Certain crosses of non-pathogenic sector lines with pathogenic lines [*normal*] gave 4-spore asci, whose 4 lines showed the same pathogenic reactions as the pathogenic parental line." Five of the nine cultural mutants of this organism so far studied (not all data have been published) induce ascospore abortion, a feature that has retarded progress of the genetic studies. Three of these five (including *white*) are pinkish white to white on malt agar; the other two are dark and not greatly different from a *normal* line in color on malt agar.

Of the genes studied, only *white* and *tan* were found to be linked. These are shown to be located on the same arm of the chromosome, 6.0 and 10.8 crossover units, respectively, from the centromere. Discovery of more linkage groups would be of interest in relation to the number of chromosomes of this species, which has not yet yielded to determination by cytological methods.

Different cultural mutants may have different effects on the expression of pathogenicity. The *tan* mutant, as shown by Keitt et al. (8), completely suppresses [4] any macroscopic pathogenic expression in every isolate-variety combination studied. Likewise, *small* suppresses macroscopic expression of pathogenicity, but as shown in plate 1, *B*, the fungus is able to penetrate and develop microscopically in the subcuticular position. As reported by Keitt et al. (8), *non-conidial* suppresses macroscopic expression of pathogenicity to all varieties studied except McIntosh, in which the sporulating-lesion reaction is reduced to a fleck. In the *white* mutant, as reported herein, there is suppression of macroscopic expression of pathogenicity of the C 5 *normal* line from which it arose as a sector; but, in the progeny stemming from a cross involving this mutant with *normal*, *white* does not affect the pathogenic reaction, though white instead of the typical dark conidia are produced on sporulating lesions. The basic reactions, sporulating lesion or fleck, are not at all affected. No explanation of this behavior of the *white* mutant based on experimental evidence can

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[4] In this paper a gene is said to suppress the expression of pathogenicity when there is no evidence of pathogenicity in the lines in which the gene occurs, whereas there is normal expression of pathogenicity in the lines from which the gene has been segregated. The statement that a gene suppresses the expression of pathogenicity or determines any other character is not intended to imply that no other genes influence the expression of the character concerned.

now be advanced, but it is probable that modifying factors in the C 5 sector (*white*) line interacting with *white* suppressed the expression of pathogenicity. If so, it would follow that these modifiers segregated into the *normal* lines in ascus XI, since the *white* lines in that ascus are pathogenic.

These demonstrations that gene mutations are capable of complete or partial suppression of pathogenicity point to the importance of the elimination of modifying factors before a genetic analysis of pathogenicity is attempted.

SUMMARY

Four mutant genes and the sex-reaction factors in *Venturia inaequalis* (Cke.) Wint. have been located with reference to their centromeres. All characters studied were found to be independently inherited except *tan* and *white*. These two genes are located on the same chromosome arm, with *tan* 10.8 and *white* 6.0 cross-over units from the centromere.

The effects of a single mutation on the behavior of the fungus may be varied and profound. The *white* mutant prevents the development of the characteristic dark pigment in the thallus, induces abortion of ascospores, and probably by interaction with modifying factors suppresses the expression of pathogenicity in the line from which it arose. Another mutant, *small*, is shown to suppress macroscopic expression of pathogenicity, but penetration of the host is accomplished and a microscopic subcuticular development occurs.

The importance of giving adequate attention to the problem of modifying factors in parental material before undertaking an analysis of the inheritance of pathogenicity is emphasized.

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INHERITANCE OF REACTION TO SMUT, STEM RUST, AND CROWN RUST IN FOUR OAT CROSSES [1]

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INTRODUCTION

In the United States, the four major diseases of oats (*Avena* spp.) and their causal pathogens are loose smut (*Ustilago avenae* (Pers.) Jens.), covered smut (*U. levis* (Kell. and Sw. Magn.), stem rust (*Puccinia graminis avenae* Eriks. and Henn.), and crown rust (*P. coronata avenae* Eriks.). The most satisfactory and in some instances the only way of controlling these diseases is by using resistant varieties. New disease-resistant varieties of oats possessing high yielding ability and other desirable agronomic characters are being produced rapidly through plant breeding. Knowledge concerning the mode of inheritance of disease reaction greatly facilitates such work. The investigations reported here were undertaken to determine the mode of inheritance of reaction to the above diseases in certain crosses involving red oats.

MATERIAL AND METHODS

The four crosses used in this study were made in the greenhouse at Manhattan, Kans., during the winter of 1939–40. Richland-Fulghum (Kans. 6155) was used as one of the parents in each of the crosses. The other parents were Anthony-Bond (Iowa 1826), Fulghum-Victoria (C. I.[3] 3485), and Fultex (C. I. 3531). Fultex is a selection from a cross between Fulghum and Victoria. These parents derived from hybrids were advanced lines that were presumably homozygous for agronomic characters and reaction to disease. Their disease reactions to the physiologic races of the rust and smut organisms used in this study are shown in table 1. The crosses studied were Richland-Fulghum × Fulghum-Victoria and reciprocal, Fultex × Richland-Fulghum, and Anthony-Bond × Richland-Fulghum.

TABLE 1.—*Reaction of parent lines to the physiologic races of the smut, stem rust, and crown rust organisms used as inoculum in studies on the inheritance of reaction to disease, Manhattan, Kans., 1941–42*

Parent line or variety	Identification No.	Inoculum							
		Ustilago avenae and U. levis		*Puccinia graminis avenae*				*P. coronata avenae*	
		Composite of races		p. r.[1] 2		p. r. 8		p. r. 1	
		Percent infection	Response	Infection type	Response	Infection type	Response	Infection type	Response
Richland-Fulghum	Kans. 6155	0	R [2]	2	R	4	S	4	S
Fulghum-Victoria	C. I. 3485	0	R	4	S	(3)		2	R
Fultex	C. I. 3531	0	R	4	S	(3)		2	R
Anthony-Bond	Iowa 1826	5.0	M R	2	R	2	R	0	R

[1] p. r.=physiologic race.
[2] R=resistant, M R=moderately resistant, S=susceptible.
[3] Not tested for reaction to physiologic race 8.

The F_1 plants were grown in the greenhouse at Manhattan, Kans., during the winter of 1940–41. Part of the seed from each F_1 plant was sent to the Aberdeen Substation, Aberdeen, Idaho, where part of the F_2 generation was grown under irrigation for seed increase purposes. The remainder was inoculated by dusting the seed with a composite of chlamydospores of several races of *Ustilago avenae* and *U. levis* known to attack the varieties Kanota and Richland and of a race of *U. avenae* known to attack the variety Fulton in Kansas. These races probably were representative of the smut organisms existing in Kansas. This seed was then space-planted in the disease nursery at Manhattan. It is recognized that, from a purely genetic point of view, the use of composite inoculum containing several physiologic races of two species of *Ustilago* is open to criticism. However, the present study was designed for its plant breeding as well as its genetic aspects. From the standpoint of practical plant breeding, resistance to all races and species was the objective. Fortunately the results obtained can be explained on a relatively simple genetic basis.

Artificial epiphytotics of crown rust and stem rust were produced in the Manhattan disease nursery by the hypodermic-syringe method of inoculating susceptible plants in uniformly distributed check and rust-spreader rows. Spore suspensions were injected into the curled leaves of developing plants. Crown rust inoculations were begun 2 weeks in advance of the stem rust inoculations. Race 1 of *Puccinia coronata avenae* and race 2 of *P. graminis avenae* were used in all of the field investigations since they were the most common races of these organisms occurring in Kansas. Each F_2 plant grown at Manhattan was examined for crown rust, stem rust, and smut at the proper time and the reaction recorded. The reactions of the resistant and susceptible parents were used as a basis for classification.

The F_3 generations studied consisted of the progeny of the F_2 plants grown at Manhattan and also at Aberdeen. The rust reactions of the F_3 generations were studied in the seedling stage in the greenhouse

during the winter and in the adult plant stage in the field the following spring. In the greenhouse each F_3 line was planted in a 2½-inch flowerpot at the rate of approximately 30 seeds per pot. Separate plantings were made for crown rust and stem rust studies. The recorded seedling reaction was that of the primary leaf only. Seedlings were inoculated 7 days after planting by placing the plants in a moist chamber, spraying them with water, and then dusting with spores. A 24-hour period in the moist chamber at ordinary greenhouse temperatures of 70° to 75° F. was found to be sufficient for infection by either rust organism. Approximately 7,000 pots of seedlings were inoculated and observed during the winter. Three pots of each parent were planted and inoculated with each lot of hybrids, giving a constant check on the intensity of inoculation and the race purity of the rust cultures. Race 1 of *Puccinia coronata avenae* and races 2 and 8 of *P. graminis avenae* were used in the greenhouse investigations. Extreme care was used in keeping all of the rust cultures pure. Differential varieties were inoculated with each race at 2-week intervals during the studies to check race purity.

Approximately 30 seeds of each F_3 line were inoculated with the smut composite and planted in the disease nursery in 1942. Smut inoculation consisted of adding spores to the seed envelopes and shaking vigorously. The seed was not dehulled. Stem rust and crown rust epiphytotics were produced in the same manner and with the same races of the rust organisms as described for the F_2 field studies in 1941. Satisfactory readings were obtained on crown rust and smut reaction but stem rust infection was neither sufficiently heavy nor distributed uniformly enough for satisfactory records in 1942.

EXPERIMENTAL RESULTS

INHERITANCE OF REACTION TO SMUTS

Studies on the inheritance of reaction of oats to the smut organisms have been made by several investigators and the reported results have indicated one, two, or three factors governing resistance, depending on the varieties used as parents. Some of the recent studies involving the varieties Bond and Victoria are particularly pertinent to the present studies. Hayes, Moore, and Stakman [4] and Hayes [5] reported that the smut reactions of the crosses Bond × Anthony and Bond × Iogold were determined by one factor with resistance dominant. The action of two dominant factor pairs, one a factor for high resistance, the other a factor for partial resistance, was observed by Torrie [6] in the crosses Iowa 444 × Bond, Victoria × Richland, and (Victoria-Richland) × State Pride.

The results reported here on the inheritance of resistance to smut agree with published results in some respects but differ from them in others. Although all of the parental lines except Anthony × Bond were known to be highly resistant to smut in Kansas, seed for

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the F_2 grown at Manhattan and for the F_3 from F_2 plants grown at Aberdeen and Manhattan was inoculated and sown in the nursery. The seed for F_2 was dehulled before smutting but seed for F_3 was smutted without dehulling. Check rows of the parents were grown at regular intervals in the nursery each year. The seed was inoculated and grown in the same manner as the hybrid lines. At heading time definite segregation was noted in both F_2 and F_3 despite the fact that both parents of all but one cross were highly resistant. F_2 plants wholly or partially smutted were classified as smutted, whereas in F_3 a line was considered smutted if one or more smutted plants appeared in the row. Only F_3 lines yielding no smutted plants were classified as resistant. In F_2 all but four of the smutted plants were only partially smutted so it was possible to grow F_3 lines from them.

Higher infection was obtained in F_3 lines from F_2 plants grown at Manhattan than in lines from similar plants grown at Aberdeen. The first three crosses had an average of 45.8 percent of the F_3 lines infected when grown from Manhattan seed and 32.4 percent from Aberdeen seed. The F_3 lines of the fourth cross had 43.3 percent and 17.5 percent infection, respectively. The reason for this is not definitely known. It was noted that seed grown under irrigation at Aberdeen was much larger and plumper than that grown under dry conditions at Manhattan. It also was noted both in the greenhouse and in the field that seedlings grown from Aberdeen seed were much more vigorous than those grown from Manhattan seed. It is possible that the poorer vigor of seedlings from Manhattan seed was associated with higher smut infection. A more important factor probably was the difference in smut conditions under which the F_2 plants were grown. The seed planted at Aberdeen was not smutted and the F_2 plants at that station were smutfree. At Manhattan the seed for F_2 was smutted and there were some smutted plants in the population. In addition, there was heavy smut in susceptible check rows of Kanota oats grown with the F_2 at Manhattan. This may have resulted in much natural infection in the field. Such possibilities suggest that the Manhattan F_3 lines probably give a more complete record of the actual inheritance of smut reaction than do lines from Aberdeen seed since there was less escape from infection at Manhattan. The data obtained from the Manhattan lines, therefore, were used in calculating the manner of inheritance, and the data on this factor are recorded in table 2.

TABLE 2.—*Segregation for reaction to smut noted in F_2 and F_3 generations of 4 oat crosses grown in the field at Manhattan, Kans.*

Cross	F_2 reaction		F_3 reaction	
	Plants observed	Plants smutted	Lines observed	Lines smutted
	Number	*Percent*	*Number*	*Percent*
Richland-Fulghum×Fulghum-Victoria	104	9.6	104	55.8
Fulghum-Victoria×Richland-Fulghum	338	3.6	337	41.8
Fultex×Richland-Fulghum	133	10.5	133	48.1
Summary of 3 R–F×F–V crosses	575	6.26	574	45.8
Anthony-Bond×Richland-Fulghum	940	1.1	938	43.3

The parental lines Fulghum-Victoria, Fultex, and Richland-Fulghum were highly resistant to the smut composite, showing no

infection. The F_2 and F_3 generations of the crosses involving these resistant parental lines showed transgressive segregation for smut susceptibility. The F_2 generations of the three crosses involving Richland-Fulghum and Fultex or Fulghum-Victoria averaged 6.26 percent smutted plants. In the F_3, 45.8 percent of the lines of these crosses grown from Manhattan seed contained smutted plants.

Apparently each parent carries a separate dominant factor pair for resistance to smut. The presence of either or both of these factors in heterozygous or homozygous condition results in resistance to smut. The factors, although different, produce the same result and therefore are considered duplicate factors. A ratio of 15 resistant to 1 susceptible plant would be expected in F_2, only those hybrid plants lacking both dominant factors being susceptible. If this hypothesis is correct, 6.25 percent of the F_2 population would be expected to be smutted; actually 6.26 percent were observed. In the F_3, 56.25 percent of the lines would be expected to show smutted plants. On this basis 7 F_3 lines would breed true for resistance, 8 would segregate (and therefore would contain smut), and 1 would breed true for susceptibility to smut. Actually 55.8 percent of the lines in 1 of the crosses were smutted. Lower percentages of infection in the other crosses probably can be accounted for by escape from smut infection as there were only 15 to 30 plants representing each line and the seed was not dehulled. The data and discussion presented concerning the crosses involving Fulghum-Victoria, Fultex, and Richland-Fulghum indicate that smut resistance in these crosses is dominant and due to 2 main factor pairs.

The Anthony-Bond parent was moderately resistant to smut, showing less than 5 percent infection, while the Richland-Fulghum parent showed no infection to the smut inoculum used. The F_2 generation had 1.1 percent of the plants smutted while 43.3 percent of the F_3 lines were smutted. These results are similar to those obtained in the other three crosses. However, in the Anthony-Bond $\times$ Richland-Fulghum cross it is assumed that there are two dominant factor pairs for resistance, one of which is a factor for high resistance, the other a factor for moderate resistance.

<div align="center">INHERITANCE OF REACTION TO STEM RUST</div>

Resistance to stem rust has been found by many investigators to be dominant and inherited on a single factor basis. Recent reports on the inheritance of resistance in cases involving the Bond and Victoria varieties are particularly pertinent to the studies reported herein. Hayes, Moore, and Stakman [7] and Hayes [8] found that reaction to *Puccinia graminis avenae* (physiologic race 1) was governed by one factor with resistance dominant in seven crosses when Bond was the susceptible parent. Torrie [9] observed similar inheritance in eight crosses involving susceptible Bond and Victoria when inoculated with races 2, 5, and 7 of *P. graminis avenae*.

The mature plant reactions of the F_2 generation in the field and the seedling reactions of the F_3 generation in the greenhouse to race 2 of *Puccinia graminis avenae* were studied at Manhattan for the crosses Richland-Fulghum $\times$ Fulghum-Victoria, Fulghum-Victoria

[7] See footnote 4, p. 45.
[8] See footnote 5, p. 45.
[9] See footnote 6, p. 45.

× Richland-Fulghum, and Fultex × Richland-Fulghum. A summary of the segregation for stem rust reaction in the F_2 and F_3 generations is given in table 3. The F_2 data are based entirely on plants grown at Manhattan; the F_3 data are based on the progeny of F_2 plants grown both at Manhattan and at Aberdeen.

The Fulghum-Victoria and Fultex parents were susceptible to race 2. Uredia on these susceptible parents were large, linear, and erumpent, producing many spores (fig. 1). On the other hand, the resistant Richland-Fulghum parent had small, circular, inconspicuous uredia which produced relatively few spores. The F_1 plants were almost as resistant as the Richland-Fulghum parent. Two types of rust reaction were observed in the F_2 plants. One phenotype resembled the resistant Richland-Fulghum parent while the other was as susceptible as the Fulghum-Victoria and Fultex parents. Of 575 plants of the 3 crosses grown in the field at Manhattan, 407 were resistant and 168 were susceptible. Resistance therefore appears to be dominant in the F_2 and to be due to a single main-factor difference. The F_3 progeny of these plants were tested in the seedling stage in the greenhouse. With but 8 exceptions the progeny from susceptible F_2 plants bred true for susceptibility in the F_3 generation. Seedlings of 33 segregating F_3 lines of Fulghum-Victoria × Richland-Fulghum and 14 segregating lines of Fultex × Richland-Fulghum were classified for rust reaction. The counts (table 3) revealed segregation closely approximating the 3 to 1 ratio observed in F_2.

The data presented indicate that in these crosses the resistance of Richland-Fulghum to physiologic race 2 is dominant and due to a single main-factor pair.

The fourth cross in which resistance to stem rust was studied was Anthony-Bond × Richland-Fulghum. The primary purpose in making this cross was to obtain segregates having combined resistance to physiologic races 2 and 8 of *Puccinia graminis avenae* and race 1 of *P. coronata avenae*. Both parents of the cross proved to be resistant to race 2 while Anthony-Bond was resistant and Richland-Fulghum was susceptible to race 8. Physiologic race 2 was used to inoculate F_2 plants in the field at Manhattan. No segregation was obtained, all of the plants being as resistant as the two parents. Inoculations with physiologic race 8 were made only on seedling plants of the F_3 generation in the greenhouse because that race has not been common in natural infections in the vicinity of Manhattan. The reaction of Anthony-Bond to physiologic race 8 was characterized by very small circular pustules surrounded by "green islands" of chlorophyll. Richland-Fulghum was susceptible to this race as shown by the presence of large, linear pustules. Figure 2 shows the seedling reaction of the parents and of hybrid lines of this cross to physiologic race 8 of *P. graminis avenae*.

By growing the progeny of F_2 plants produced both at Manhattan and Aberdeen, 1,272 F_3 lines were available for study. Of this number, 324 lines were observed to be breeding true for resistance, 632 lines were segregating, and 316 lines were breeding true for susceptibility. These were close to the expected numbers on the basis of a single factor difference (table 3). Counts also were made in 52 of the segregating lines and revealed a ratio of 1,811 resistant to 579 susceptible plants.
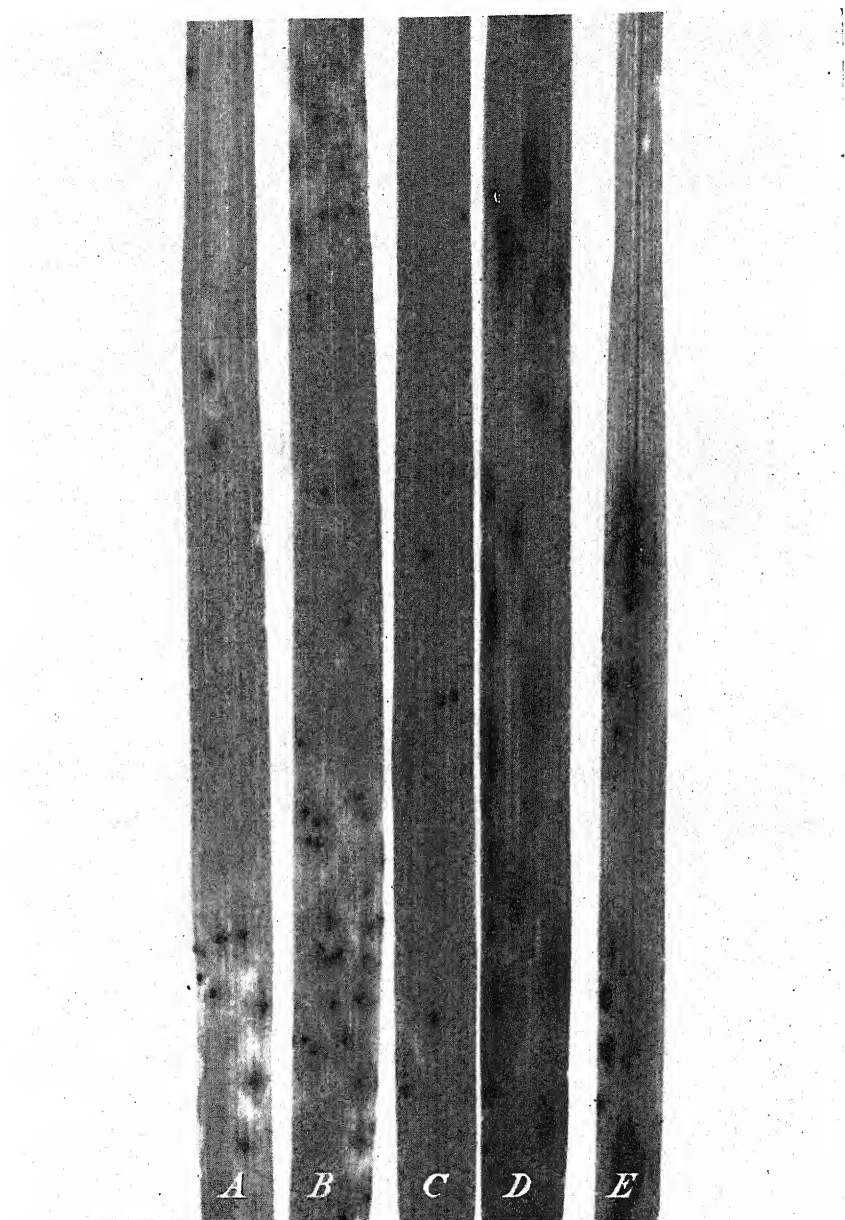
FIGURE 1.—Reaction of primary leaves of seedling plants to *Puccinia graminis avenae* physiologic race 2 in the cross Richland-Fulghum × Fulghum-Victoria and in the two parental lines: *A*, resistant Richland-Fulghum parent; *B* and *C*, resistant F₃ lines; *D*, a susceptible F₃ line; *E*, the susceptible Fulghum-Victoria parent.
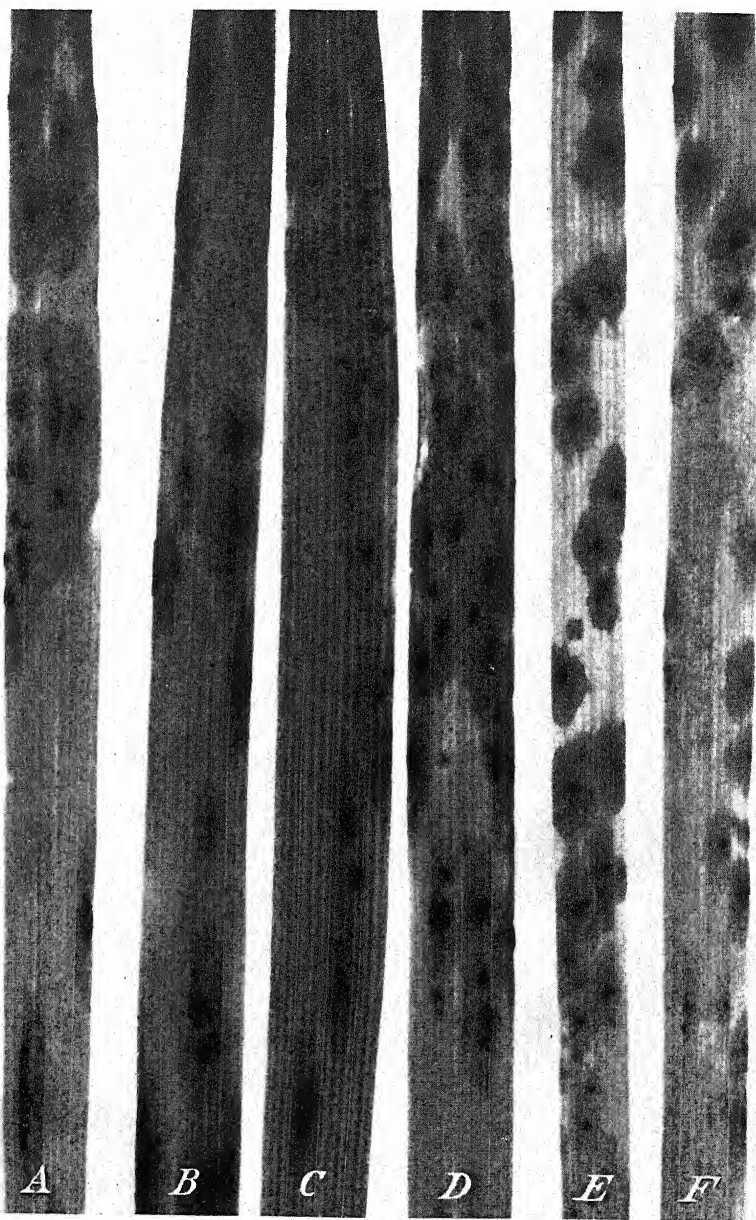
FIGURE 2.—Reaction of primary leaves of seedling plants of the cross Anthony-Bond × Richland-Fulghum and of parental lines to *Puccinia graminis avenae* physiologic race 8: *A*, susceptible Richland-Fulghum parent; *B*, a susceptible F₃ line; *C* and *D*, types of intermediate F₃ lines; *E*, a resistant F₃ line; *F*, the resistant Anthony-Bond parent.

TABLE 3.— *Segregation for reaction to Puccinia graminis avenae in 4 oat crosses in mature F_2 plants in the field and seedlings of F_3 lines grown in the greenhouse; the first 3 crosses were inoculated with physiologic race 2 and the last with race 8*

Cross	Observed or expected	F₂ generation in field — Number of plants R	S	Total	χ²	Range of P	F₃ generation in greenhouse — Number of lines R	Seg.	S	Total	χ²	Range of P	Segregation in segregating F₃ lines — Number of lines counted	Number of plants R	S	Total	χ²	Range of P
Richland-Fulghum × Fulghum-Victoria	O	73	31	104	1.282	0.20–0.30	22	65	33	120	2.850	0.20–0.30						
	E	78	26				30	60	30									
Fulghum-Victoria × Richland-Fulghum	O	237	101	338	4.024	.01–.05	151	346	227	724	17.370	.01	33	1,104	385	1,489	0.606	0.30–0.50
	E	253	85				181	362	181					1,117	372			
Fultex × Richland-Fulghum	O	97	36	133	.363	.50–.95	66	186	101	353	7.878	.01–.05	14	482	163	645	.033	.50–.95
	E	100	33				88	177	88					484	161			
Summary of R–FX, F–V crosses	O	407	168	575	5.336	.01–.05	239	597	361	1,197	24.903	.01	47	1,586	548	2,134	.490	.30–.50
	E	431	144				299	599	299					1,600	534			
Anthony-Bond × Richland-Fulghum	O	940	0	940			324	632	316	1,272	.151	.50–.95	52	1,811	579	2,390	.723	.30–.50
	E						318	636	318					1,793	597			

It appears, therefore, that resistance to race 8 of stem rust is dominant and is controlled in this cross by one factor which differs from that governing resistance to race 2 in the other crosses.

INHERITANCE OF REACTION TO CROWN RUST

Murphy, Stanton, and Stevens,[10] and Weetman [11] reported that the inheritance of the crown rust resistance of Victoria is controlled by one dominant genetic factor. The inheritance of the crown rust resistance of Bond was found to be governed by two dominant complementary factors by Hayes, Moore, and Stakman,[12] Hayes,[13] and Weetman.[11] Torrie [14] suggested that the crown rust reaction in the cross Iowa No. 444 × Bond was governed by two factor pairs, one a factor for crown rust resistance and the other a partial inhibitor of the factor for resistance.

The reaction of the F_2 generation of all four crosses to race 1 of *Puccinia coronata avenae* was studied at flowering time in the field in 1941. The reaction of the F_3 generation of the same crosses was studied in the seedling stage in the greenhouse during the following winter and in the mature plant stage in the field in 1942. Pure cultures of physiologic race 1 were used in all inoculations.

A strain of Richland-Fulghum was used as the susceptible parent in each of the four crosses. This strain has large normal uredia unaccompanied by chloronemia. The Fulghum-Victoria and Fultex parents were resistant, having small, nearly circular pustules surrounded by the necrotic areas so characteristic of the Victoria type of resistance. Severe seedling infection of this type caused a complete drying of the infected leaves of resistant plants, giving the seedlings a blighted appearance. Typical seedling reactions of the parents and of the F_3 hybrids of the crosses involving the Victoria type of resistance are shown in figure 3. Anthony-Bond, the resistant parent of the fourth cross, exhibited the near-immunity of Bond.

Since the two types of resistance to crown rust race 1 proved to be inherited in different ways, they will be discussed separately. The manner of inheritance of the Victoria type of resistance will be considered first. The F_1 plants were nearly as resistant as the Fulghum-Victoria and Fultex parents. Three different types of rust reaction were observed in mature plants of the F_2 generation in the field.

One phenotype resembled the resistant parent, another resembled the susceptible parent, and a third type was intermediate, duplicating the F_1 reaction. Owing to their near-resistance the intermediate types and resistant types were grouped together and compared with the susceptible. Reaction of the F_2 and F_3 generations determined on that basis are shown in table 4. In the F_2 generation, segregation appeared to be 3 resistant plants to 1 susceptible, while the F_3 segregated 1 resistant line, to 2 segregating lines, to 1 susceptible line. Thus

[10] MURPHY, H. C., STANTON, T. R., and STEVENS, H. BREEDING WINTER OATS RESISTANT TO CROWN RUST, SMUT, AND COLD. Amer. Soc. Agron. Jour. 29 : 622–637. 1937.
[11] WEETMAN, L. M. GENETIC STUDIES IN OATS OF RESISTANCE TO TWO PHYSIOLOGIC RACES OF CROWN RUST. (Abstract) Phytopathology 32 : 19. 1942.
[12] See footnote 4, p. 45.
[13] See footnote 5, p. 45.
[14] See footnote 6, p. 45.
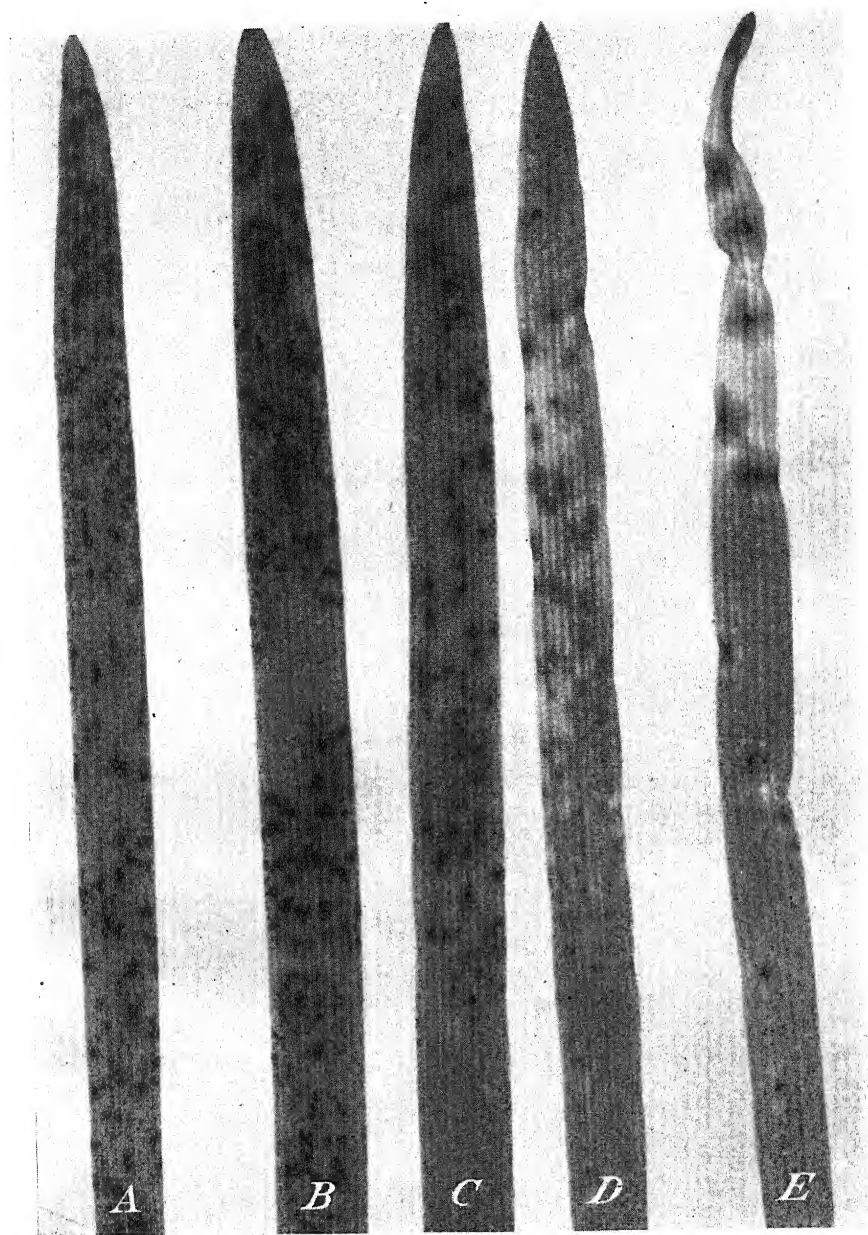
FIGURE 3.—Reaction of primary leaves of seedling plants of the cross Richland-Fulghum × Fulghum-Victoria and of parental lines to physiologic race 1 of *Puccinia coronata avenae: A*, the susceptible Richland-Fulghum parent; *B*, a susceptible F₃ line; *C*, an intermediate F₃ line; *D*, a resistant F₃ line; *E*, the resistant Fulghum-Victoria parent.

resistance to crown rust in the three crosses involving Fulghum-Victoria and Fultex was partially dominant and controlled by one factor. There was good agreement between field and greenhouse F_3 observations and there appeared to be no difference in the crown rust reaction of seedlings and mature plants in the progeny of these crosses.

TABLE 4.—*Segregation in 3 oat crosses for reaction to Puccinia coronata avenae physiologic race 1 observed in the mature F_2 plants in the field and seedlings of F_3 lines grown in the greenhouse*

Cross	Observed or expected	F₂ generation in field					F₃ generation in greenhouse					
		Number of plants			x^2	Range of P	Number of lines				x^2	Range of P
		R	S	Total			R	Seg	S	Total		
Richland-Fulghum × Fulghum-Victoria	O	65	39	104	8.667	0.01	31	45	44	120	10.317	0.01
	E	78	26				30	60	30			
Fulghum-Victoria × Richland-Fulghum	O	258	80	338	.393	.50- .95	178	368	178	724	.199	.50- .95
	E	253	85				181	362	181			
Fultex × Richland-Fulghum	O	100	33	133	.000	1.00	76	173	104	353	4.636	.05- .10
	E	100	33				88	177	88			
Summary of R-F × F-V crosses	O	423	152	575	.593	.30- .50	285	586	326	1,197	3.376	.10- .20
	E	431	144				299	599	299			

The inheritance of resistance to crown rust in Anthony-Bond × Richland-Fulghum was different from that encountered in the other crosses. Anthony-Bond carries the high resistance of Bond to most of the prevalent races of *Puccinia coronata avenae* including physiologic race 1. The only symptom of crown rust infection observed in hybrid and parental plants carrying that type of resistance was a slight flecking where infection occurred (fig. 4).

Plants in F_1 exhibited at the four- to five-leaf stage an intermediate reaction. In F_2 it became evident that the inheritance of resistance in this cross was more complex than that found in the other crosses. F_2 plants were grown in the field and crown rust readings were made at flowering time. The plants were divided into resistant and susceptible groups. The resistant group was subdivided into two more or less distinct classes, one of which was highly resistant, showing only slight flecking, while the other was moderately resistant, exhibiting slight pustule development and heavy flecking. The susceptible group also was subdivided into two groups, one fully susceptible and the other intermediate but approaching full susceptibility. In many cases it was difficult to determine whether a plant should be classed as fully susceptible or as intermediate. A similar difficulty sometimes was experienced in distinguishing between highly resistant and moderately resistant plants. There were 310 plants of the F_2 generation classified as resistant. Of this group 120 plants were classed as highly resistant and 190 as moderately resistant. In the susceptible group there were 630 plants, 158 of which were classed as intermediate and 472 as fully susceptible (table 5).
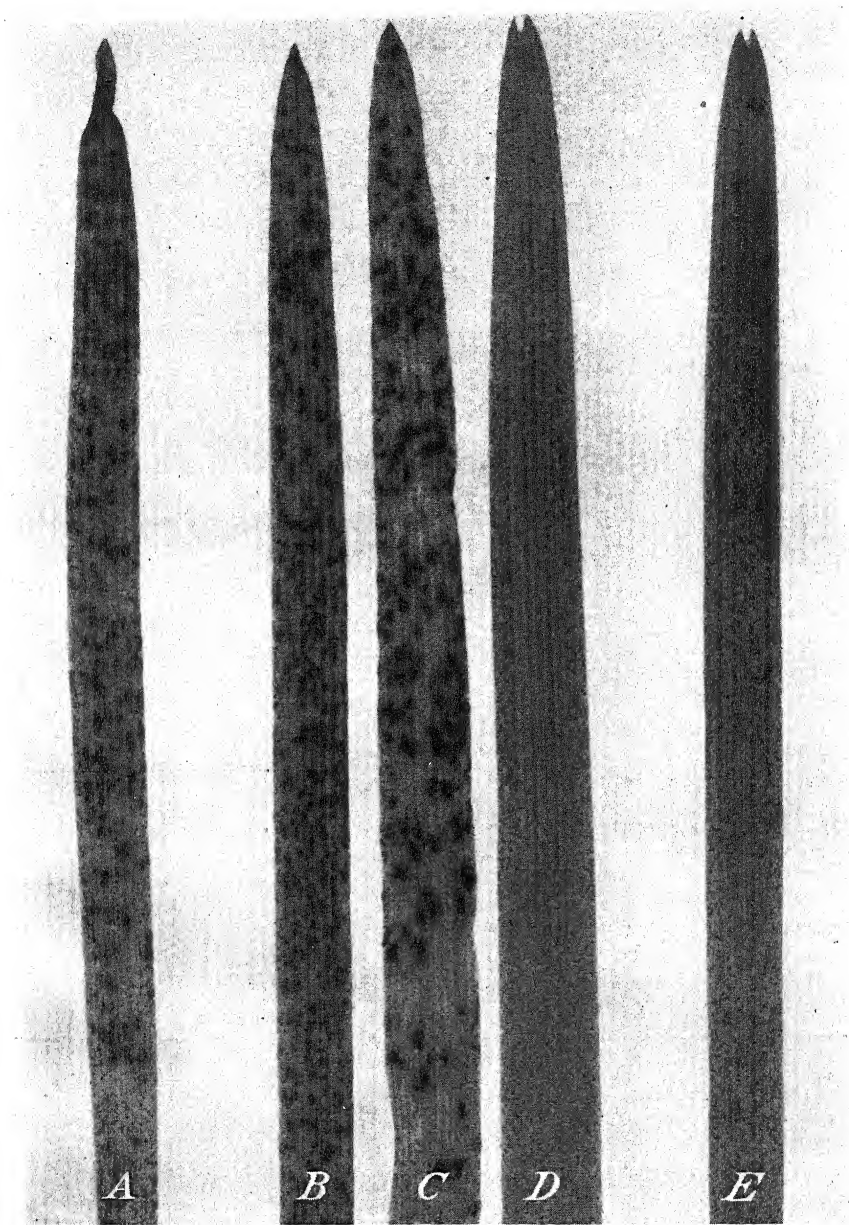
FIGURE 4.—Reaction of primary leaves of seedling plants of the cross Anthony-Bond × Richland-Fulghum and of parental lines to physiologic race 1 of *Puccinia coronata avenae*: *A*, the susceptible Richland-Fulghum parent; *B* and *C*, susceptible F₃ lines; *D*, a resistant F₃ line; *E*, the resistant Anthony-Bond parent.

TABLE 5.—*Reaction to crown rust of 940 F₂ plants of the cross Anthony-Bond ✕ Richland-Fulghum grown in the field at Manhattan, Kans., and the breeding behavior of seedlings of the F₃ lines tested with Puccinia coronata avenae in the greenhouse*

Reaction of F₂ plants [1]	Reaction of F₃ lines [2]		
	Highly resistant	Segregating	Susceptible
	Number	*Number*	*Number*
Highly resistant (120)_____	27	93	0
Moderately resistant (190)_____	6	183	1
Intermediate (158)_____	0	150	8
Susceptible (472)_____	0	63	409
	33	489	418

[1] χ^2 for F₂ data based on ratio of resistant to susceptible classes 0.121, range of P 0.50–0.95.
[2] χ^2 for F₃ data based on ratio of highly resistant: segregating: susceptible 3.512, range of P 0.10–0.20.

While segregation for reaction to crown rust in F_2 was determined by readings made only on adult plants grown in the field, the F_3 was studied both in the seedling stage in the greenhouse and in the flowering stage in the disease nursery. The most striking difference between the F_2 segregation in the field and readings on F_3 seedlings in the greenhouse was the complete absence of moderately resistant and intermediate types in the greenhouse. F_3 seedlings were inoculated in the primary-leaf stage of growth and they were either highly resistant or fully susceptible. This simplified greenhouse observations since the differences were so apparent.

In addition to the 940 F_2 plants grown at Manhattan, 1,051 plants were grown at Aberdeen. Thus 1,991 F_3 lines were tested for seedling reaction to crown rust in the greenhouse. Of this number, only 50 lines bred true for resistance, 989 segregated, and 952 bred true for

TABLE 6.—*Breeding behavior of F₃ lines of the cross Anthony-Bond ✕ Richland-Fulghum when inoculated with Puccinia coronata avenae in the greenhouse, compared with the reaction of flowering plants of the same lines to the same rust in the field*

Location	Stage of growth	Observed or expected	F₃ lines				χ^2	Range of P
			Resistant	Segregating	Susceptible	Total		
			Number	*Number*	*Number*	*Number*		
Field_____	Flowering_____	O	50	1,002	939	1,991	0.337	0.50–0.95
Greenhouse_____	Seedling_____	O	50	989	952	1,991	.474	.50– .95
		E	54	996	941	1,991		

susceptibility (table 6). In segregating lines all types of segregation, from those containing mostly resistant plants to those containing mostly susceptible plants, appeared.

The F_3 field and greenhouse reactions were similar in that the same lines bred true for resistance and susceptibility and the same lines segregated in the greenhouse and in the field (table 6). However, moderately resistant and intermediate types appeared in segregating lines in the field. By making counts in certain segregating lines in the field it was concluded that the moderately resistant types were

highly resistant in the seedling stage while the intermediate types were fully susceptible. The inheritance of resistance to crown rust in this cross is explained on a four-factor basis in which two sets of complementary factors were interacting. A factorial analysis of resistant and segregating lines based on this hypothesis is shown in table 7.

TABLE 7.—*Factorial analysis of homozygous resistant and segregating lines in F_3 of the cross Anthony-Bond × Richland-Fulghum showing seedling and flowering plant reactions to Puccinia coronata avenae expected on the basis of a 4-factor difference* [1]

	Phenotypic expression [2] in—			
F₂ genotype	Seedling plants		Flowering plants	
	F₂ plant	F₃ line	F₂ plant	F₃ line
AABBCCDd	S	1R:3S	I	1R:2I:1S.
AABBCcDD	S	1R:3S	I	1R:2I:1S.
AABBCcDd	R	11R:5S	MR	7R:4MR:4I:1S.
AABbCcDD	S	3R:13S	I	3R:6I:7S.
AaBBCcDD	S	3R:13S	I	3R:6I:7S.
AABbCCDd	S	3R:13S	I	3R:6I:7S.
AaBBCCDd	S	3R:13S	I	3R:6I:7S.
AABbCcDd	R	33R:31S	MR	21R:12MR:12I:19S.
AaBBCcDd	R	33R:31S	MR	21R:12MR:12I:19S.
AaBbCCDd	S	9R:55S	I	5R:4MR:18I:37S.
AaBbCcDD	S	9R:55S	I	5R:4MR:18I:37S.
AaBbCcDd	S	83R:173S	I	35R:48MR:52I:121S.
AABBCCdd	R	R	R	R.
AABBCcdd	R	R	R	R.
AaBBCCdd	R	3R:1S	R	3R:1S.
AABbCCdd	R	3R:1S	R	3R:1S.
AaBBCcdd	R	3R:1S	R	3R:1S.
AABbCcdd	R	3R:1S	R	3R:1S.
AaBbCCdd	R	9R:7S	MR	5R:4MR:7S.
AaBbCcdd	R	9R:7S	MR	5R:4MR:7S.
AABBccDD	R	R	R	R.
AABBccDd	R	R	R	R.
AaBBccDD	R	3R:1S	R	3R:1S.
AABbccDD	R	3R:1S	R	3R:1S.
AaBBccDd	R	3R:1S	R	3R:1S
AABbccDd	R	3R:1S	R	3R:1S.
AaBbccDD	R	9R:7S	MR	5R:4MR:7S.
AaBbccDd	R	9R:7S	MR	5R:4MR:7S.
AABBccdd	R	R	R	R.
AaBBccdd	R	3R:1S	R	3R:1S.
AABbccdd	R	3R:1S	R	3R:1S.
AaBbccdd	R	9R:7S	MR	5R:4M7S.

[1] *A* and *B* are complementary factors for resistance carried by Anthony-Bond. *C* and *D* are complementary inhibitors of the expression of *A* and *B* and are carried by Richland-Fulghum. Only 32 genotypes are shown. The remaining 49 bred true for susceptibility.
[2] The symbols R, MR, I, and S are phenotypic symbols denoting high resistance, moderate resistance, intermediacy, and susceptibility, respectively.

Since the four phenotypic classes appeared only in the reaction of mature plants, the following discussion of gene interaction applies to the mature plant. It is assumed that two dominant complementary factors (A and B) for resistance are carried by the Anthony-Bond parent. These factors are inherited independently and, because of their complementary nature, both must be present to give a plant the expression of the Bond type of resistance. When both of these factors are heterozygous ($AaBb$), the plant is not highly resistant but is only moderately resistant in the adult stage. However, if both dominant factors are homozygous ($AABB$), or if one is homozygous and the other heterozygous ($AABb$ or $AaBB$), the plant shows the Bond type of resistance to crown rust. Plants breeding true for resistance

must be homozygous for both dominant factors ($AABB$). If either or both of these factors are recessive ($aaBB, aaBb, AAbb, Aabb, aabb$) the plant is fully susceptible and will breed true for susceptibility.

The inheritance in the cross studied would be relatively simple if only the two complementary factors A and B were involved. However, the inheritance has been complicated by the action of two other independently inherited dominant complementary factors, C and D from the Richland-Fulghum parent, which when present together, are capable of inhibiting the expression of the factors for resistance. The degree to which they inhibit the expression of resistance is determined by two conditions: (1) The number of dominant inhibiting genes present, and (2) the number of dominant genes for resistance present. It is possible for two, three, or four dominant inhibiting genes to be present and show an inhibiting effect, depending on whether the plant is heterozygous for both ($CcDd$), heterozygous for one ($CcDD, CCDd$) or homozygous for both ($CCDD$). If homozygous for both, four dominant inhibiting genes are acting, masking the expression of any combination of genes for resistance and giving a fully susceptible reaction. The progeny of any plant which carries both of the inhibitors in a dominant homozygous condition will breed true for susceptibility to crown rust regardless of the genes for resistance (A and B). Thus $AABBCCDD$ would breed true for susceptibility.

If a plant is heterozygous for one inhibitor and homozygous for the other ($CCDd$ or $CcDD$), it carries three dominant inhabiting genes whose inhibiting effect on any combination of the genes for resistance gives an intermediate-type reaction when both genes for resistance are present.

If a plant is heterozygous for both inhibitors ($CcDd$), the inhibitors show their weakest inhibiting effect because only two domiant genes are present. The inhibitors in this doubly heterozygous condition change the expression of the genes for high resistance ($AABB, AaBB, AABb$) to that of moderate resistance in the mature plant. The interaction of these four genes all in a heterozygous condition ($AaBbCcDd$) results in an intermediate reaction in mature plants.

Since the moderately resistant and intermediate types appeared among mature plants of the F_2 and F_3 generations in the field and did not appear in the seedling plants of a similar F_3 generation in the greenhouse, it follows that the types appearing as moderately resistant or intermediate in the field appeared either as highly resistant or susceptible in the greenhouse where a sharp segregation occurred. In order to determine how these genotypes were expressed phenotypically in the seedlings, the rust reactions of about 100 selected segregating F_3 lines were observed in the seedling and in the mature-plant stage. From these observations it was concluded that all types appearing as moderately resistant in the mature plant appeared as highly resistant in the seedling. Similarly, all types appearing as intermediate in the field appeared as susceptible in the seedling stage.

Segregation of the F_2 generation in the field on the proposed four-factor hypothesis would theoretically result in a ratio of 35 highly resistant plants : 48 moderately resistant : 52 intermediate : 121 susceptible. When placed on a basis of the 940 plants actually grown: a ratio of 129 resistant: 176 moderately resistant: 191 intermediate: 444 susceptible would be expected. Actually a ratio of 120 resistant:

190 moderately resistant : 158 intermediate : 472 susceptible was observed. When the resistant and moderately resistant plants were placed in one class and the intermediate and susceptible in another class, a ratio of 310 resistant to 630 susceptible plants was obtained as shown in table 5. The observed F_2 ratio, therefore, closely fits the expected ratio as shown by the χ^2 test.

According to the foregoing hypothesis, only 54 of the F_3 lines would be expected to breed true for resistance. Actually, 50 lines were observed in both the greenhouse and the field to be breeding true for resistance (table 6). Theoretically, exactly half of the population (996 lines) would be expected to segregate. In the greenhouse 989 lines appeared to segregate, while 1,002 seemed to be segregating in the field. According to the hypothesis, 941 lines were expected to breed true for susceptibility. Actually, 952 lines in the greenhouse and 939 lines in the field appeared to be breeding true for susceptibility.

The close agreement between observed and expected ratios in both F_2 and F_3 (table 5) is further substantiated by the plant counts made in segregating F_3 lines in the greenhouse. The number of resistant and susceptible plants expected in the various segregating groups can be added to give numbers which represent the segregation as a whole. If the F_3 segregating lines are considered collectively, 46.24 percent of the seedlings would be expected to be resistant and 53.76 percent susceptible. There were 12,973 plants counted in 440 consecutive segregating F_3 lines. Of this number 6,137 (47.3 percent) were resistant, and 6,836 (52.7 percent) were susceptible. The agreement between the observed and expected percentages, therefore, appears to be good considering the many different types of segregation that occurred and the great variation in the number of plants in each of the 440 segregating F_3 lines.

This four-factor hypothesis confirms the reports of Hayes, Moore, and Stakeman,[15] Hayes,[16] and Weetman,[17] who state that the crown rust reaction of Bond is controlled by two complementary factors. It also shows how a fairly simple inheritance may be complicated by the action of an additional set of complementary factors. The hypothesis is well substantiated by the close agreement between observed and expected numbers in the F_2 and F_3 generations. The analysis of 440 segregating F_3 families adds even further proof, since all expected types of segregation were observed. Certain selected lines studied in the F_4 generation segregated or bred true for susceptibility or resistance as expected.

RELATION OF CROWN RUST, STEM RUST, AND SMUT REACTION

From both scientific and practical points of view it semed desirable to ascertain whether there was any association between the factors for resistance to any of the three diseases. No relationship was noted between stem and crown rust inheritance in the Fulghum-Victoria × Richland-Fulghum and Fultex × Richland-Fulghum crosses as the observed 9 : 3 : 3 : 1 F_2 ratio was close to the expected χ^2 being 2.246 and P within the range of 0.50–0.95. In the Anthony-Bond × Richland-Fulghum cross, the x^2 on F_3 data for the expected ratio showing

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[15] See footnote 4, p. 45.
[16] See footnote 5, p. 45.
[17] See footnote 11, p. 52.

no linkage was 10.817 (8 degrees of freedom) with the range of P being 0.20–0.30.

Smut resistance appeared to be distributed at random among the various classes of rust reaction.

Victoria and Bond types of reaction to crown rust show differences in their degree of resistance. The resistance of Bond approaches immunity, showing only a slight flecking at points of infection, while the less desirable resistance of Victoria is expressed by small pustules surrounded by necrotic areas. Weetman [18] has reported that the resistance of Bond to race 1 is conditioned by two dominant complementary genes and that the resistance of Victoria to the same race is due to one factor distinct from the Bond genes.

This investigation has confirmed Weetman's report in regard to the nature and number of factors involved in the inheritance of each type of resistance. Since both types of resistance were not involved in any one of the four crosses studied in this investigation it was impossible to determine whether the two types of resistance were inherited independently. However, some knowledge of this was gained by observing the F_2 segregation of certain crosses, Fultex × Bond-Fulghum and Fultex × D 69-Bond, that were grown in the disease nursery in 1942. Three main types of crown rust reaction were observed. Approximately 37 percent of the plants had the Victoria type of resistance, 49 percent had the Bond type of resistance, and 14 percent were susceptible. The respective percentages expected would be 33, 56, and 11, if it is assumed that the three factors involved were independently inherited and that the Bond type masked the Victoria type of resistance when all three dominant factors were present in the F_2 plants. Therefore the data obtained seem to indicate that the factor determining Victoria resistance is distinct from the two governing the resistance of Bond.

SUMMARY

The inheritance of reaction to a mixture of races of *Ustilago avenae* and *U. levis*, *Puccinia graminis avenae* physiologic races 2 and 8 and *P. coronata avenae* physiologic race 1 was studied in four crosses, Richland-Fulghum × Fulghum-Victoria and reciprocal, Fultex × Richland-Fulghum, and Anthony-Bond × Richland-Fulghum in the field and in the greenhouse. The disease reaction of the first three crosses was the same, but that of the fourth cross was different because of the genetic constitution of the Anthony-Bond parent.

Transgressive segregation for smut susceptibility occurred in the first three crosses. The parents of these crosses were all highly resistant to the smut inoculum, showing no infection. The reaction of the hybrids in the F_2 and F_3 generations indicated that each parent carried a dominant factor for resistance. When these two independently inherited duplicate dominant factors were both absent in a hybrid, the plant was susceptible to smut.

In the Anthony-Bond × Richland-Fulghum cross a similar condition existed except that there was one dominant factor for high resistance to smut and one for moderate resistance.

[18] See footnote 11, p. 52.

Resistance to stem rust was dominant in all four crosses and appeared to be governed by a single factor.

Reaction of the three crosses involving Fulghum-Victoria and Fultex to crown rust was governed by one factor with resistance dominant.

The crown rust reaction of Authony-Bond × Richland-Fulghum was controlled by the interaction of four factor pairs, two sets of dominant complementary factors. One set, dominant complementary genes for resistance, was carried by Anthony-Bond, the resistant parent, and the other set, dominant complementary inhibitor genes epistatic to the genes for resistance was carried by Richland-Fulghum, the susceptible parent. The expected F_2 ratio was 35 fully resistant : 48 moderately resistant : 52 intermediate : 121 fully susceptible plants. In the seedling stage in the greenhouse the moderately resistant plants were fully resistant, while the intermediate plants were fully susceptible. The expected F_3 segregation was 7 lines breeding true for resistance, 128 lines segregating, and 121 lines breeding true for susceptibility. Observed ratios in F_2 and F_3 proved to be statistically near the expected ratios.

No association between the factors determining the reaction to different diseases was observed in any of the crosses studied.

EFFECTS OF TWO MUSTARD OILS ON PLASMODIOPHORA BRASSICAE AND THEIR RELATION TO RESISTANCE TO CLUBROOT [1]

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INTRODUCTION

The possible effects of mustard oils on the development of clubroot (*Plasmodiophora brassicae* Wor.) of crucifers were first suggested by Rochlin (Rokhlina) (*14*, 15).[3] She concluded that resistance was associated with the presence of glucosides yielding mustard oils upon hydrolysis. When cabbage (*Brassica oleracea* var. *capitata* L.) seedlings grown in heavily infested soil were watered with the aqueous extracts of seeds of black mustard (*B. nigra* (L.) Koch), only 20 percent became diseased, while in untreated controls 100 percent were infected. The toxic properties of mustard oils to various bacteria and fungi have been demonstrated (*3, 6, 7, 11, 13, 19*).

When Pryor (*12*) grew turnip (*Brassica rapa* L.), black mustard, and cabbage in sand cultures without sulfur in the nutrient solution the plants were found to be very low in mustard-oil content, yet their relative resistance or susceptibility to clubroot was only slightly altered. He concluded that factors other than mustard oils were chiefly responsible for resistance. Stahmann, Link, and Walker (*17*) isolated beta-phenethyl isothiocyanate from the root tissues of resistant and susceptible strains of turnip and black mustard, from a susceptible strain of white mustard (*B. hirta* Moench (*B. alba* (L.) Boiss.)), and from susceptible cabbage, but the presence of allyl isothiocyanate could not be detected. The roots of horseradish, (*Armoracia rusticana* Gaertn., Mey., and Scherb.) contained both beta-phenethyl isothiocyanate and allyl isothiocyanate. These investigators were unable to correlate resistance with either mustard-oil content or myrosin activity in these hosts.

The investigation reported here consisted of a study of the effects of the two mustard oils, allyl isothiocyanate ($CH_2\!=\!CH\!-\!CH_2\!-\!N\!=\!C\!=\!S$) and beta-phenethyl isothiocyanate ($C_6H_5\!-\!CH_2\!-\!CH_2\!-\!N\!=\!C\!=\!S$), on the germination and infectivity of the clubroot organism.

MATERIALS AND METHODS

Since *Plasmodiophora brassicae* is an obligate parasite and since clubroot tissue is rapidly invaded by secondary organisms, it is difficult to obtain suspensions of resting spores entirely free from other

organisms. In these investigations spore suspensions were purified as much as possible after the method described by Wellman (*20*). Firm clubs from diseased plants were washed, frozen at −4° C., and stored in the frozen state until used. Spores were separated from host debris by first macerating diseased tissue in a meat chopper or Waring blendor and then filtering through cheesecloth with the spores remaining in the filtrate. Small pieces of host tissue passing through the cheesecloth were separated from the spores by centrifuging for about 15 seconds. The supernatant liquid containing spores was then centrifuged in a Sharples supercentrifuge. The puttylike mass of spores was suspended in distilled water and the process repeated until the spores had been carried through at least three complete changes of relatively large volumes of water. Purified spore suspensions were either held overnight at 8° C., and diluted the following day for spore germination studies, or frozen and held until used. Throughout the series of investigations, unless otherwise specified, the resting-spore concentration was maintained at approximately 25,000 spores per cubic millimeter.

Mustard-oil solutions were prepared from stocks obtained in 1938 and stored continuously at 8° C. Allyl isothiocyanate, practical grade, was purchased from a camera company; beta-phenethyl isothiocyanate was synthesized after the method described by Stahmann, Link, and Walker (*17*). Because of the low solubility of the phenethyl oil in water, mustard-oil solutions were prepared by the addition of aliquots of a stock solution of oil in ethyl alcohol to distilled water. Since these oils react slowly with ethyl alcohol, stock solutions were prepared and stored at 8° C. generally 12 hours before the final dilution.[4] The final ethyl-alcohol concentration, never exceeding 1 percent by volume, was usually 0.7 percent. Equal volumes of mustard-oil solutions as well as spore suspensions prepared at twice the desired concentrations were thoroughly mixed, resulting in spore suspensions and oil solutions of a given concentration.

The effects of mustard oils on zoospore formation were observed in aqueous mustard-oil solutions of 50 ml. contained in 125 ml. Erlenmeyer flasks tightly stoppered with a tin-foil-covered cork. Flasks were stored in the dark at 23° C. Daily observations of zoospores were made by means of a Levy blood-counting chamber. The flasks were vigorously shaken before sampling and a drop of spore suspension was removed from the solution with a 5-mm. loop of nichrome wire. Zoospore counts were made by determining the number of zoospores in 15 fields each containing $1/250$ mm^3. Five fields were examined from each sample and three samples were removed from each flask.

The effects of the two oils upon *P. brassicae* were determined in a different manner by the amount of infection and disease development on cabbage, which has been shown to be very susceptible to the organism (*18*). Healthy cabbage plants, 3 to 4 inches high, of the yellow-resistant variety Jersey Queen, were planted into clubroot-free, moist, muck soil which had been adjusted to pH 5.4. The soil was first packed into 4-inch clay pots and five holes per pot formed in the soil with a glass rod to facilitate planting. A 5-ml. aliquot of spore

suspension in mustard-oil solution was placed with the plant in each of the five transplanting holes. The soil was firmly packed around the plants and well watered with distilled water. Since approximately 18 hours of high soil moisture is necessary for infection (*20*), the soil was kept as moist as possible for 3 to 4 days until infection had been established. To preclude excessive damping-off, the soil moisture was then gradually reduced and maintained at as low a level as was still compatible with good growth. The plants were grown for about 2 months, then removed and examined.

EXPERIMENTAL RESULTS

EFFECTS OF MUSTARD OILS ON GERMINATION OF PLASMODIOPHORA BRASSICAE

The effects of allyl and of beta-phenethyl isothiocyanate on spore germination were determined by direct zoospore counts. The course of spore germination in this study was in general agreement with the findings of Wellman (*20*), who observed that the rate of germination in water cultures gradually increased to a peak on the fourth day of incubation, after which it gradually decreased. Very little germination was observed during the first 2 days of incubation. In this study the time at which germination in distilled water reached a peak varied from the third to the fifth day, and sometimes the peak extended over a period from the fourth to the seventh day. It was usually impossible to make accurate zoospore counts after the seventh day because of the development of bacteria, protozoa, and filamentous fungi in the culture solution. Since it was necessary to use a small amount of ethyl alcohol in the preparation of the oil suspensions, controls were prepared in 0.7 percent solution of the former, a concentration slightly greater than that to which the spores were generally exposed. Since no consistent effect of alcohol upon germination was observed in the trials conducted, it was concluded that the effect of mustard oils upon germination was independent of the alcohol used in the preparation of the oil.

In the high concentrations the addition of mustard oils to the aqueous suspensions of spores was observed to have a marked inhibitory effect upon germination. The toxic levels at which spore germination was inhibited by each oil are shown in table 1. Original concentrations of the allyl oil inhibiting spore germination of *P. brassicae* ranged from 10 to 80 p. p. m., whereas original concentrations of beta-phenethyl isothiocyanate inhibiting visible germination ranged from 5 to 80 p. p. m. Whenever direct comparisons of the toxic levels of the oils could be made, no consistent difference was evident. In experiment 2, the phenethyl oil was more toxic than the allyl oil and in experiment 8 the reverse obtained. In all other trials the oils were approximately equal in toxicity when compared on a weight basis. However, since the beta-phenethyl oil is 1.7 times the molecular weight of the allyl oil, the former was almost twice as toxic when compared on a molecular basis.

In addition to the inhibitory effect exercised by the oils, marked stimulation of zoospore production was observed at oil concentrations below the inhibitory levels. The results of a typical experiment (experiment 2, table 1) in which direct comparisons could be made between the two oils on zoospore formation are shown in

TABLE 1.—*Effects of 2 mustard oils upon germination of the resting spores of Plasmodiophora brassicae, as determined by direct zoospore counts*

Experiment No.	Initial concentration completely inhibiting spore germination		Initial concentration stimulating spore germination	
	Isothiocyanate		Isothiocyanate	
	Allyl	Phenethyl	Allyl	Phenethyl
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*
1	20.0	----------	10.0	----------
2	10.0	5.0	2.5	1.25
3	10.0	----------	1.25–5.0	
4	80.0	80.0	.6–10.0	1.25–10.0
5	80.0	(1)	40.0	(2)
6	80.0	80.0	40.0	(2)
7	20.0	20.0	10.0	(2)
8	40.0	80.0	20.0	40.0

[1] Toxic concentration not clearly defined.
[2] Germination not sufficient to indicate most favorable concentration.

figures 1 and 2. The allyl oil (fig. 1) was decidedly stimulatory to germination at initial concentrations of 1.25 and 2.5 p. p. m. The higher concentration more nearly approached the optimum in this experiment although good germination took place at both levels. On the other hand, it is evident that the phenethyl oil was toxic at 2.5 p. p. m., while at 1.25 p. p. m. it was more stimulatory than was the allyl form at 2.5 p. p. m.

The extent of germination and the level at which stimulation was greatest varied considerably from one experiment to another. This may have been due in part to variability in the spore suspensions
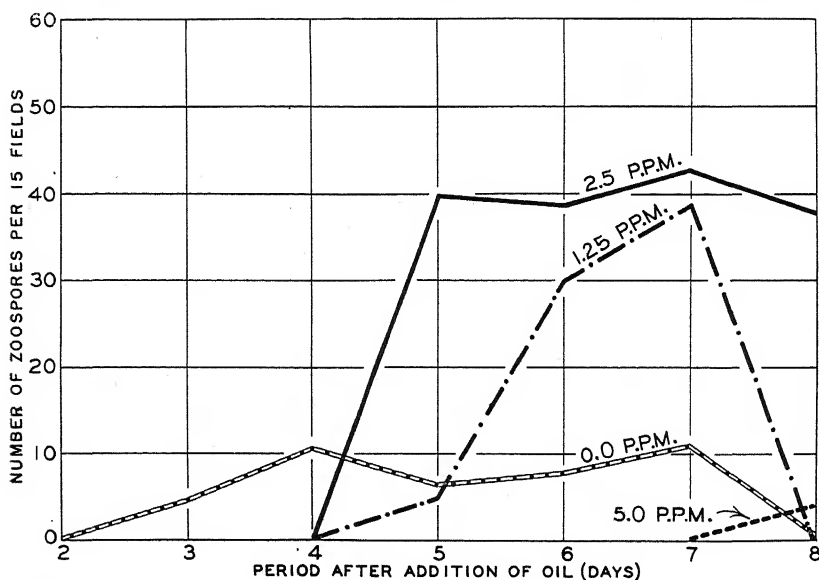
FIGURE 1.—Effect of allyl isothiocyanate at different concentrations on the germination of resting spores of *Plasmodiophora brassicae.* No germination was observed in this experiment at concentrations of 10 p. p. m. and higher.

which, although made up by uniform procedures, were from diseased plants collected at different times and in different locations. The beta-phenethyl oil stimulated germination at concentrations below the toxic level in three out of six trials, while the allyl was effective in each trial. However, when the former caused stimulation, the effect was more pronounced than with the allyl oil.
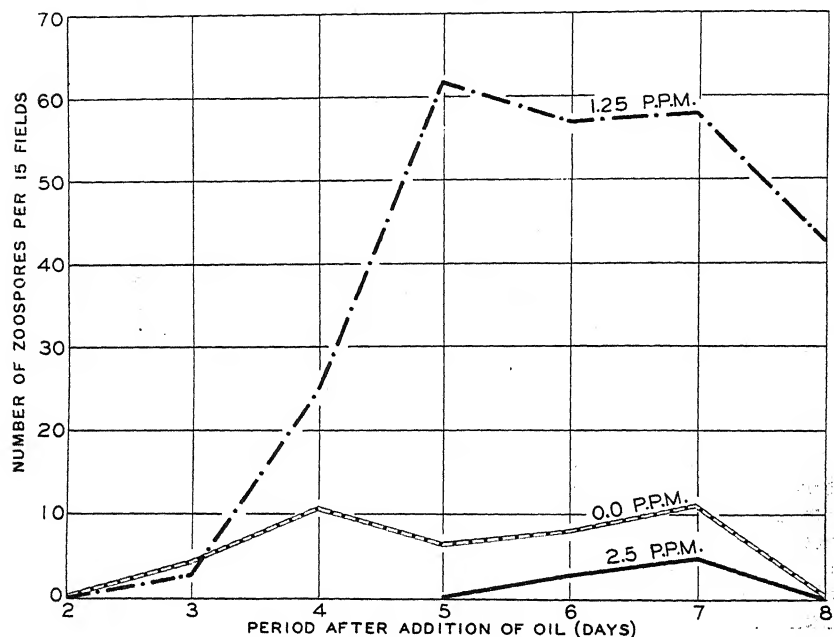
FIGURE 2.—Effect of beta-phenethyl isothiocyanate at different concentrations on the germination of resting spores of *Plasmoidophora brassicae*. No germination was observed in this experiment at concentrations of 5 p. p. m. and higher.

EFFECTS OF ALLYL ISOTHIOCYANATE ON COLLETOTRICHUM CIRCINANS AT SUBLETHAL LEVELS

Since the mustard oils stimulated spore germination of *Plasmodiophora brassicae*, a limited number of tests were conducted with a readily culturable fungus, *Colletotrichum circinans* (Berk.) Vogl., in order to determine whether or not the reaction to the oils was peculiar to *P. brassicae*. This organism was selected because the spores germinate readily in distilled water and because its reaction to mustard oils had been determined previously (7, 13, 19). Since the stimulatory effect on *P. brassicae* occurred more consistently with allyl isothiocyanate than with the beta-phenethyl oil, tests on germination of spores *C. circinans* were carried out with the first-mentioned oil.

Spore suspensions were prepared after the method of McCallan and Wilcoxon (9). Approximately 10 ml. of water was added to spores which had been gently scraped from the surface of an agar slant. The suspension was at once poured off into a sterile tube, filtered through a small amount of absorbent cotton, and centrifuged for 10 minutes after which the supernatant liquid was poured

off. The spores were taken up in distilled water and stored at 80° C. until used. Petri dishes adapted as moist chambers were used. Sterile glass slides were placed on U-shaped glass rods placed in the bottom of the dish. Approximately 2.5 ml. of oil and water of the desired concentration was placed in the bottom of the Petri dish. This oil reserve served to maintain a supply of oil vapor in the moist chamber during the period of germination. Oil solutions and spore suspensions at twice the desired concentration were thoroughly mixed and a 5-mm. loop of the suspension was placed on the glass slide. The Petri dishes were placed inside large moist chambers and stored at 23° C. until the spores had germinated.

The limitations of moist chambers of this type for the study of a volatile chemical are apparent. However, it was believed that a suitable range of concentrations was maintained over the relatively short period required for germination even though the initial concentration may have dropped as a result of the evaporation of oil. The ethyl alcohol concentration throughout the trials was maintained at 1.0 percent by volume.

Spore germination in 1.0 percent ethyl alcohol was generally very similar to that in distilled water, indicating that the solvent had little effect in the final reaction. Observations on spore germination made at the end of 5 hours showed no stimulatory effect of allyl isothiocyanate at subinhibitory concentrations upon spore germination. Previous trials, the results of which are not presented, had shown no stimulatory effect upon spore germination at subinhibitory levels after 12 hours' incubation. Allyl isothiocyanate in these incompletely closed systems prevented germination in all cases at concentrations of 40 p. p. m., and usually lower concentrations were inhibitory, indicating that in general *C. circinans* was more sensitive than *P. brassicae* to allyl isothiocyanate (table 2).

TABLE 2.—*Germination of spores of Colletotrichum circinans in drops of allyl isothiocyanate solution*

Concentration (p. p. m.)	Germination [1]		
	Experiment 1	Experiment 2	Experiment 3
	Percent	*Percent*	*Percent*
0.0 [2]	70	59	
0.0 [3]	71	58	87
0.002	75	63	88
0.004	74	51	86
0.008	64	62	84
0.019	76	60	
0.039	66	57	82
0.078	75	56	78
0.156	70	58	74
0.312	74	58	80
0.625	71	43	82
1.25	·65	59	80
2.5	40	33	38
5.0	6	26	2
10.0	2	0	0
20.0	3	0	0
40.0	0	0	0

[1] Figures represent the percent germination of 100 spores after 5 hours' incubation.
[2] Control prepared with distilled water only.
[3] Control and all mustard-oil solutions prepared in 1.0 percent ethyl alcohol.

EFFECTS OF MUSTARD OILS ON DISEASE DEVELOPMENT

The effect of mustard oils on infection and disease development as reported by Rochlin (*14, 15*) has already been mentioned. Comparative studies on the toxicity of the two oils on disease development were carried out as previously described on cabbage plants grown in the greenhouse.

MUSTARD-OIL INJURY TO CABBAGE

In addition to the toxic effects of the mustard oils to *Plasmodiophora brassicae*, it was found that considerable injury to the host resulted at certain concentrations. Typical injury is shown in figure 3. Affected plants exhibited symptoms typical of damping-off be-
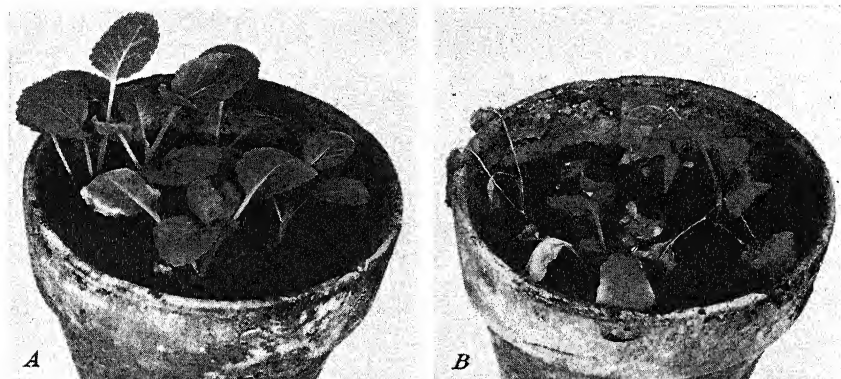
FIGURE 3.—Cabbage plants showing effect of beta-phenethyl isothiocyanate. *A*, plants transplanted with a liquid containing 300 p. p. m.; no injury resulted. *B*, plants transplanted with a liquid containing 400 p. p. m.; marked injury resulted.

cause of the rapid deterioration of the lower stem and subsequent collapse of the plant. Occasionally plants were able to develop roots above the point of injury and survive. Recovered plants could be readily recognized when removed from the soil by the complete absence of the main root or by the protrusion of the stump of the original root system and lower stem from a tuft of actively developing roots above the point of injury. When mixed with spore suspensions of *P. brassicae* the allyl oil caused some injury to cabbage at 100 p. p. m., while the phenethyl oil was toxic at 350 and 400 p. p. m.

TOXICITY OF MUSTARD OILS TO SPORES USED AS INOCULUM

The relative toxicity of the two mustard oils to the resting spores of *Plasmodiophora brassicae* was determined, infection and disease development of cabbage being used as an indication of spore inactivation. Spores were usually mixed with the oil 2 days before the suspension was used as inoculum; in experiments 1 and 5 (table 3) the exposure period was 3 and 4 days, respectively.

In certain instances, cabbage plants that had been protected from infection by the addition of oil to the inoculum showed greater vegetative growth than those severely diseased after inoculation with spore suspensions in oil solutions at subinhibitory concentrations. Dif-

TABLE 3.—*Relative toxicity of allyl and beta-phenethyl isothiocyanate when mixed with a spore suspension of Plasmodiophora brassicae used as inoculum*

Oil concentration (P. p. m.)	Percent of cabbage plants infected at concentrations (p. p. m.) indicated [1]											
	Experiment 1		Experiment 2		Experiment 3		Experiment 4		Experiment 5		Experiment 6	
	Allyl	Phenethyl	Allyl	Phenethyl	Allyl	Phenethyl	Allyl	Phenethyl	Allyl	Phenethyl	Allyl	Phenethyl
0.0	100	100	100	100	100	100	100	100	93	80	73	92
1.25					100	100	----	----	87	60	92	82
2.5					67	100	100	20	93	80	75	91
5.0					28	62	27	43	53	64	80	93
10.0					8	92	14	53	100	53	78	83
20.0					0	8	23	7	80	29	43	40
30.0					0	0	0	0	0	0	12	33
50.0	100	100	50	33	0	0	0	0	0	0	0	55
75.0	80	73	8	43	----	----	0	0	0	0	0	0
100.0	0	33	0	13	----	----	0	0	0	0	0	0
150.0	13	100	0	29	----	----	0	0	0	0	0	0
200.0	0	77	0	0	----	----	0	0	0	0	0	0
250.0	0	7	0	23	----	----	0	0	----	----	0	0
300.0	0	0	0	31	----	----	0	0	0	0	0	0
350.0			0	55	----	----	0		----	----		0
400.0			0	0	----	----	0		0	0	----	0

[1] 15 plants were inoculated with each suspension.

ferences of this nature are shown in figure 4. Infection was precluded by a transplanting solution containing 75 p. p. m. of allyl isothiocyanate (*B*); whereas 100 percent disease development took place, when a solution containing 50 p. p. m. was used (*A*).

The infectivity of the inoculum with and without removal of the oil at the end of the period of exposure was compared to determine whether the end results secured in table 3 were the effect in part of the carry-over influence of oil added with the suspension at the time of transplanting. It was possible to remove practically all of the oil by centrifugation. The results of three experiments are given in table 4.
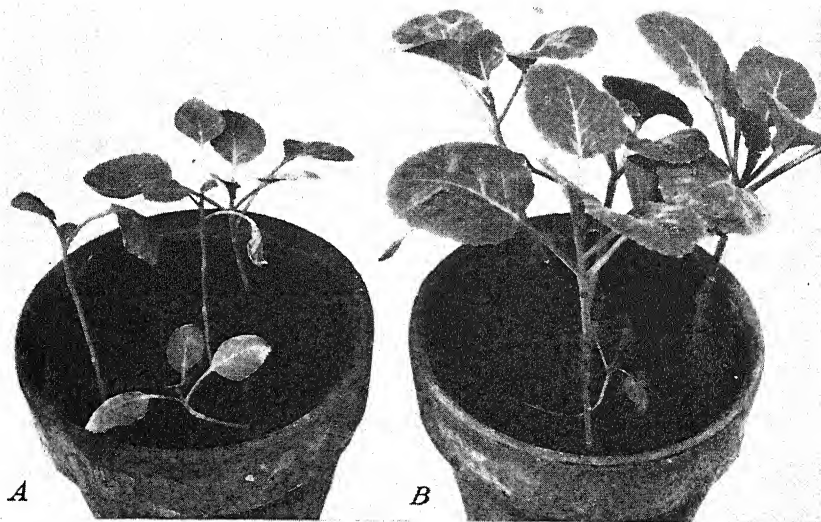
FIGURE 4.—Effect on clubroot development of two concentrations of allyl isothiocyanate in spore suspensions used as transplanting liquids. *A*, at 50 p. p. m. infection was complete and disease severe. *B*, at 75 p. p. m. no infection occurred.

TABLE 4.—*Relation of time of exposure of Plasmodiophora brassicae to mustard oils and the removal of oil from the inoculum, to the infectivity of the spore suspension*

Experiment No.	Oil used	Days exposed to oil	Oil present or absent in inoculum	Percent of cabbage plants infected at concentrations (p. p. m.) indicated [1]															
				0	1.25	2.5	5	10	20	30	50	75	100	150	200	250	300	350	400
1	Allyl	3	Present	100	—	—	—	—	—	—	100	80	0	13	0	0	0	0	—
		3	Absent	100	—	—	—	—	—	—	86	0	7	0	0	0	0	0	7
		10	Present	100	—	—	—	—	—	—	73	33	0	36	23	47	14	13	0
		10	Absent	100	—	—	—	—	—	—	100	58	8	0	0	0	0	0	0
		24	Present	100	—	—	—	—	—	—	93	20	53	100	77	0	0	55	0
		24	Absent	100	—	—	—	—	—	—	100	87	33	93	93	7	0	31	0
	Phenethyl	3	Present	100	—	—	—	—	—	—	100	73	0	100	36	57	0	0	0
		3	Absent	100	—	—	—	—	—	—	0	36	93	100	0	8	0	7	0
		10	Present	100	—	—	—	—	—	—	100	100	60	0	10	0	0	0	0
		10	Absent	100	—	—	—	—	—	—	100	47	100	33	0	7	0	0	0
		24	Present	93	—	—	—	—	—	—	100	100	0	0	0	0	0	0	0
		24	Absent	83	—	—	—	—	—	—	50	8	71	0	0	22	31	0	0
2	Allyl	2	Present	100	—	—	—	—	—	—	29	45	0	0	0	0	0	—	0
		2	Absent	76	—	—	—	—	—	—	0	0	0	29	0	0	0	—	0
		10	Present	93	—	—	—	—	—	—	14	0	0	86	85	0	0	—	0
		10	Absent	100	—	—	—	—	—	—	0	0	13	0	15	23	31	—	0
		23	Present	55	—	—	—	—	—	—	0	43	27	0	0	23	7	—	0
		23	Absent	50	—	—	—	—	—	—	33	46	0	0	0	0	27	—	0
	Phenethyl	2	Present	100	—	—	—	—	—	—	0	7	0	0	0	0	0	—	0
		2	Absent	93	—	—	—	—	—	—	33	41	0	0	0	0	0	—	0
		10	Present	100	—	—	—	—	—	—	0	0	0	0	0	0	0	—	0
		10	Absent	100	—	—	—	—	—	—	0	0	0	0	0	0	0	—	0
		23	Present	80	—	—	—	—	—	—	0	0	7	0	0	0	5	—	0
		23	Absent	100	—	—	—	—	—	—	0	0	9	0	9	0	12	—	0
5	Allyl	4	Present	93	87	93	53	100	80	0	—	—	—	—	—	—	—	—	—
		4	Absent	100	92	100	89	100	73	100	—	—	—	—	—	—	—	—	—
		14	Present	100	92	100	89	100	92	100	—	—	—	—	—	—	—	—	—
		14	Absent	80	100	86	100	53	90	93	—	—	—	—	—	—	—	—	—
	Phenethyl	4	Present	93	60	80	64	100	29	0	—	—	—	—	—	—	—	—	—
		4	Absent	100	93	100	100	100	73	14	—	—	—	—	—	—	—	—	—
		14	Present	90	90	100	100	100	100	0	—	—	—	—	—	—	—	—	—
		14	Absent	100	100	92	100	100	91	8	—	—	—	—	—	—	—	—	—

[1] 15 plants were inoculated with each suspension.

There was a tendency for the toxic effect of each oil to increase with length of exposure but it did not hold consistently with either one. There was also a tendency for the inoculum to be infective over a wider range of concentrations when the oil was removed before inoculation, indicating that at the end of a given exposure part of the spores were only inhibited and others killed. This trend, however, was not entirely consistent. It would seem, therefore, that the comparative effects given in table 3 are a fairly accurate index of relative toxicity of the oils and that changes in length of exposure and in removal of oil would not materially change the end result.

RELATION OF SPORE CONCENTRATION TO TOXICITY

It has been shown by a number of investigators that toxic levels of chemicals may be considerably changed by different spore concentrations. Smith (*16*) reported that as the spore concentration increased in 4 percent phenol, the percentage of spores surviving exposure to the toxic substance increased. McCallan, Wellman, and Wilcoxon (*10*) observed that increasing the spore concentration in dilute copper sulfate solutions and in bordeaux mixture resulted in germination at higher concentrations of copper than was possible with lower spore concentrations. Pryor, Walker, and Stahmann (*13*) varied spore concentrations exposed to vapors of allyl isothiocyanate and observed that colony development was precluded by lower partial pressures of oil when the spore concentration had been reduced 100 times.

Trials were conducted to determine the effect of spore concentration of *P. brassicae* on the level of allyl isothiocyanate required to prevent infection in cabbage. The data (table 5) secured in three experiments showed that, in general, as the spore concentration increased from 25,000 to 800,000 spores per cubic millimeter the concentration of allyl isothiocyanate required to prevent infection progressively increased.

TABLE 5.—*Effect of increasing concentrations of Plasmodiophora brassicae spores on toxicity of allyl isothiocyanate*

Experiment No.	Spore concentration (spores per mm³.)	Percent of cabbage plants infected at concentrations (p. p. m.) indicated [1]													
		0.0	1.25	2.5	5.0	10.0	20.0	30.0	50.0	75.0	100	150	200	250	300
1	25,000	100	-----	-----	-----	-----	-----	-----	-----	0	0	0	0	0	0
	50,000	-----	-----	-----	-----	-----	-----	-----	-----	0	0	0	0	0	0
	100,000	-----	-----	-----	-----	-----	-----	-----	-----	79	0	0	0	0	0
	200,000	71	-----	-----	-----	-----	-----	-----	-----	100	36	0	0	0	0
4	25,000	100	-----	100	27	14	23	0	0	0	0	0	0	0	0
	50,000	97	-----	73	40	40	58	7	0	0	0	0	0	0	0
	100,000	100	-----	100	79	80	27	0	0	0	0	0	0	0	0
	200,000	100	-----	100	93	64	8	0	0	0	0	0	0	0	0
6	25,000	73	92	75	80	79	43	12	0	0	0	0	0	0	0
	100,000	75	75	90	100	57	64	50	44	29	0	0	0	0	0
	200,000	75	90	86	82	100	100	100	71	37	0	0	0	0	0
	400,000	100	92	73	80	91	53	50	22	20	25	0	0	0	0
	800,000	86	87	71	80	100	77	64	62	33	50	0	0	0	0

[1] 15 plants inoculated with each suspension.

It has been reported above that at certain levels the oil in the spore suspension was toxic to the host plant. As the concentration of spores increased, the concentration of oil required to cause death of the host plant progressively increased. This is illustrated in figure

5. In table 6 are given the number of surviving plants at various concentrations of oil and spores. It is apparent that at each oil level at which plant injury occurred the toxicity was usually reduced with increase in spore concentration. Thus it is evident that the spores take up the oil or detoxify it in proportion to their number per given volume of suspension. Consequently, the concentration of spores in the inoculum is important in determining whether or not a given level of oil will be sufficiently toxic to prevent infection or to cause host injury.
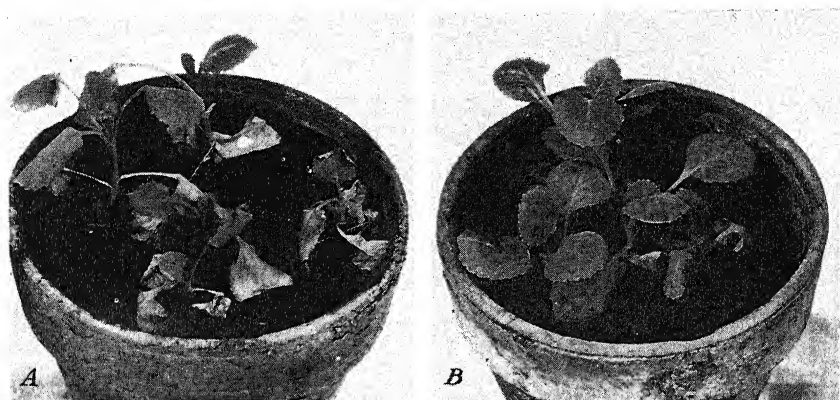
FIGURE 5.—Influence of spore concentration on the phytotoxic effect of allyl isothiocyanate in the inoculum. *A*, plants inoculated with a suspension containing 50,000 spores per cubic millimeter and 200 p. p. m. of oil; injury severe. *B*, Plants inoculated with a suspension containing the same concentration of oil but 100,000 spores per cubic millimeter; injury slight.

TABLE 6.—*Effect of increasing concentrations of Plasmodiophora brassicae spores on the survival of cabbage plants at various levels of allyl isothiocyanate*

Oil concentration p.p.m.	Number of plants surviving from 15 planted at number of spores per mm.[3] indicated—			
	25,000	50,000	100,000	200,000
0	14	--------	--------	14
75	12	14	14	15
100	12	12	14	14
150	0	12	10	13
200	3	6	9	13
250	2	5	4	7
300	0	0	8	5
350	0	0	0	1
400	0	0	0	1

EFFECTS OF MUSTARD OILS ON CLUB SIZE

It was noted in certain of the toxicity experiments described above that the clubs tended to be larger in plants exposed to subinhibitory oil concentrations containing spore suspensions than in plants developing in the absence of oil. Clubs were weighed in each trial in order to determine the effects of oils on club size. It became apparent that mustard-oil solutions at subinhibitory concentrations were often capable of favoring club development by protecting the infected

root from secondary decay.   Since in practically all trials, clubs developing in the absence of oils showed a greater tendency to rot than those exposed to oils, the influence of secondary organisms in the development of the club was a factor in club size.   However, final evaluation of the data failed to show that club size was increased by the presence of mustard oil in the inoculum.

DISCUSSION

Allyl and beta-phenethyl isothiocyanates have been shown to be highly toxic to a number of fungi (*7, 13, 19*).   The former occurs in the roots of horseradish, while the latter is present in the roots of horseradish, cabbage, black mustard, white mustard, and turnip (*17*). In the first case, and presumably in the second, they occur as glucosides rather than as free oils.   The glucoside of the allyl oil, sinigrin, has relatively little or no toxicity to fungi tested (*19*).   The possible relation of the mustard oils to resistance in certain varieties of mustard and turnip to clubroot has been the occasion of the present study of their effect upon the pathogen, *Plasmodiophora brassicae*.

The two oils were compared by adding them in various concentrations to spore suspensions of the pathogen.   When the relative number of zoospores per microscopic field was used as a criterion and when the oils were compared on a molecular basis, the phenethyl oil was generally more toxic than the allyl oil.   On the other hand, when the spore suspensions so treated were used as inocula around the roots of young cabbage transplants, the percentage of diseased plants indicated that the two oils were usually about equal in effect and that sometimes the allyl oil was the more toxic.   When the time of exposure was increased and when the oil was removed at the end of the exposure period, no consistent change in results was observed.

Both oils stimulated spore germination of *P. brassicae* at levels below the inhibitory concentrations, although allyl isothiocyanate did so more consistently.   No stimulation of spore germination of *C. circinans* was effected by allyl isothiocyanate.   It is possible that the oils are specific to *P. brassicae* in their sitmulatory properties.   Stimulation of fungi by toxic substances has been reported by a number of investigators.   Latham (*8*) showed that the vapors of chloroform stimulated mycelial growth of *Sterigmatocystis*.   Duggar (*5*) reported that ethyl alcohol and copper sulfate in low concentrations stimulated germination of certain fungus spores.   Brown (*2*) showed that volatile substances arising from bruised leaves and apple tissue stimulated germination of *Botrytis* spores, whereas substances from potato tissue, orange rind, onion leaves, and bulb scales inhibited spore germination. Brown (*1*) showed that substances liberated from plant leaves and petals into drops of water are capable of either stimulating or inhibiting germination.

The levels of allyl isothiocyanate preventing infection and disease development were shown to be influenced by variation in spore concentration.   These observations agree with results reported with various fungi by Smith (*16*), McCallan, Wellman, and Wilcoxon (*10*), and Pryor, Walker, and Stahmann (*13*).

Considerable variation between experiments was encountered throughout the investigation.   Although the exact reason for this

lack of uniformity has not been determined, it is obvious that a number of factors are concerned. Among these are the effect on the oils of secondary organisms in the root extracts; variations within the clubroot inoculum from different locations; variation in period of storage of clubbed roots; and variations in the greenhouse environment influencing infection and disease development. McCallan and Wilcoxon (9), who were able to grow their test organisms in pure culture under constant conditions, found that spores from replicate transfers varied a great deal more than would be expected from random sampling and they suggest that spores from the same tube be used in laboratory tests of fungicides in order to eliminate the error arising from the use of replicate transfers. The differences existing between different spore samples in the tests reported in this paper are undoubtedly of sufficient magnitude to account for a large part of the variability in results.

Evidence concerning the lack of importance of mustard oils in resistance to *P. brassicae* has been obtained through three different lines of approach. Pryor (12) showed that even though the mustard-oil content of resistant and susceptible strains of turnip and black mustard and of susceptible strains of cabbage was definitely reduced by low-sulfur nutrition, the inherent disease reaction was little altered. He concluded that the sulfur oils are not essential in enabling the host to prevent or retard clubroot development in its tissues. Stahmann, Link, and Walker (17) determined the mustard-oil content as well as the myrosin activity in the tissues of resistant and susceptible strains of turnip and black mustard and were unable to correlate either of these properties with resistance. In this paper it has been shown that the mustard oils, allyl and beta-phenethyl isothiocyanates, are very toxic to the clubroot organism at concentrations as low as 10 p. p. m. of the free oils. It was estimated that the beta-phenethyl oil occurred in turnip and mustard roots at concentrations ranging from 0.010 to 0.024 percent (100 to 240 p. p. m.). If the oils occurred in the free state they should be sufficiently concentrated to prevent infection. However, it has not been shown that free oil is liberated from the roots into the soil solution as has been reported for germinating cruciferous seeds by Denny (4), and if such were the case it seems unlikely that oil concentrations comparable to those combined as glucosides within the tissue could be maintained outside the plant. There is still no adequate explanation of resistance since chemical analyses (17) show about equal amounts of the oils in roots of very susceptible species such as cabbage and in both resistant and susceptible turnips and black mustard. No evidence of differential enzyme activity was found which might control the release of free oil in resistant as compared with susceptible plants (17).

It is evident both from these investigations and from earlier ones (17) that the mere presence in the host of a material highly toxic to one of its pathogens is no proof that the toxic substance functions in restriction of pathogenicity. The data presented herein would indicate quite as strongly that these oils liberated in minute amounts might even be stimulatory to *P. brassicae* and enhance infection rather than restrict it. In fact there may be some connection between the specific stimulatory effect of these oils to *P. brassicae* and the fact that its obligate parasitism is restricted to the *Cruciferae*.

SUMMARY

Direct observations on the germination of resting spores of *Plasmodiophora brassicae* Wor. exposed to allyl and beta-phenethyl isothiocyanate were made. Either oil in certain concentrations is capable of inhibiting spore germination. Little difference in the toxicity of the two oils was observed when compared on a weight basis, but when compared on a molecular basis the phenethyl oil was almost twice as toxic as allyl isothiocyanate.

When the toxicity of the oils to the clubroot organism in spore suspensions was tested by inoculation of cabbage plants, the two were most often approximately equal in toxicity, when compared on a molecular basis, although in certain instances allyl isothiocyanate was definitely more toxic than phenethyl isothiocyanate. Increasing the time of exposure of the spores to oil before planting, sometimes but not consistently resulted in an increased toxic effect of the oil. In some experiments removal of oil from the inoculum at the end of the exposure period indicated that the oil had had an inhibitive effect on some spores and a lethal effect on others.

At concentrations below the toxic level, both oils were definitely stimulatory to germination of spores, but the allyl oil stimulated germination more consistently than the beta-phenethyl isothiocyanate. The spores of *Colletotrichum circinans* (Berk.) Vogl. were not stimulated in germination by allyl isothiocyanate under the experimental conditions employed.

As the concentration of spores was increased, higher concentrations of allyl isothiocyanate were required to prevent disease development.

No consistent change in relative club size resulted from the presence of mustard oils in the spore inoculum or from exposure of spores to oil before their use as inoculum, except that at sublethal levels there was a tendency for the oil to protect the club from decay by secondary soil organisms.

Concentrations of potentially available beta-phenethyl isothiocyanate in the roots of both the resistant and susceptible crucifers examined are roughly comparable to the concentrations of oil required to prevent disease development and spore germination when mixed with spore suspensions of *P. brassicae*. However, the liberation of this oil in appreciable quantities from the roots of cruciferous plants has not been demonstrated. If it may be assumed that mustard oils are liberated from roots of crucifers into the soil solution, it seems unlikely that oil concentrations comparable to those combined as glucosides within the tissue could be maintained outside the plant. Furthermore, from what is known of the concentration of the phenethyl oil in cruciferous roots, as great protection would be expected to be afforded the susceptible varieties of turnip and mustard as is afforded the resistant varieties, since the oil content of the roots of the susceptible and resistant varieties is about the same. If oil concentrations in the soil solution outside the plant were below the toxic level, a stimulatory effect on *P. brassicae* spores could conceivably be induced. In fact it is possible that the mustard oils, if present in the proper concentration in the soil solution, might actually predispose cruciferous plants to disease rather than increase their resistance.

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RESISTANCE OF TOBACCO TO BLUE MOLD (PERONOS-PORA TABACINA)[1]

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INTRODUCTION

All varieties of tobacco (*Nicotiana tabacum* L.) grown in the United States are highly susceptible to the blue mold disease, caused by *Peronospora tabacina* Adam. Therefore, to provide a sound basis for a breeding program, a search has been made for adequate resistance to blue mold in the genus *Nicotiana*. The available material consisted of some 1,000 collections of *N. tabacum* from all parts of the world, but in particular from Mexico and Central and South America.[3] In addition, seed was available [4] from many of the wild *Nicotiana* species. The work was begun in 1933 and has been continued without interruption to the present time.

MATERIALS AND METHODS

The procedure first followed in testing for resistance to blue mold was to sow the seeds in rows (fig. 1) and expose the plants when 5 or 6 weeks old to blue mold attack. However, it was soon discovered that age and vigor of growth had a very marked effect on the susceptibility of plants to blue mold. Hence, to obtain more uniform material, the seedlings were transplanted into 2½-inch earthen pots, and great care was taken to provide optimum conditions for rapid growth. The importance of uniformly severe disease attacks was also apparent. Thus, genotypes that endured moderate blue mold attacks without serious damage might be completely killed by severe epidemics. It was impossible to provide uniformly severe conditions in plant beds; so, although plant-bed tests were used extensively, major dependence was placed on greenhouse experiments. Thus, it was found that from November to March quite uniformly severe conditions could be provided in the greenhouse, and that when potted plants 6 to 8 weeks old were used, the results corresponded very closely with those obtained in plant-bed tests under the severest conditions. The usual number of greenhouse plants used per lot was 10, and conclusions regarding susceptibility of *Nicotiana* species were based on at least 5 separate tests conducted at intervals over a period of several years. Wherever the need for maximum accuracy was apparent, as, for example, with the most resistant species and
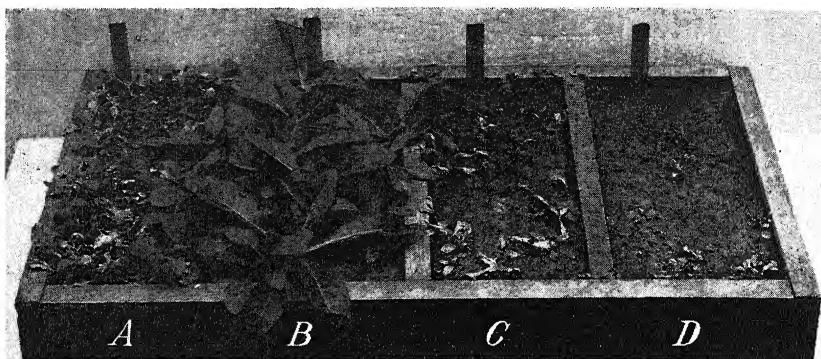
FIGURE 1.—Plants showing response of different species of *Nicotiana* to blue mold attack. *A* and *C*, Varieties of *Nicotiana tabacum; D, N. sylvestris;* all three susceptible to blue mold. *B, N. debneyi*, highly resistant. Photographed in 1934; at that time *N. debneyi* was regarded as a strain of *N. suaveolens* Lehm.

certain slightly resistant genotypes of *N. tabacum*, testing was much more extensive and prolonged. The recording of disease data was facilitated by the use of index numbers as shown in table 1.

TABLE 1.—*Index numbers used to record the amount of blue mold disease and the degree of resistance of Nicotiana species*

Index number	Amount of disease	Degree of resistance
0	No evidence of disease	Immune.
1	Small lesions; no visible sporulation	Highly resistant.
2	Moderate-size lesions; slight sporulation	Moderately resistant.
3	Large lesions and partial defoliation; abundant sporulation	Slightly resistant.
4	General defoliation; abundant sporulation; plants killed	Susceptible.

RESULTS

RESISTANCE IN NICOTIANA TABACUM

Search for resistance to blue mold in *Nicotiana tabacum* resulted in the discovery of a number of South American types, chiefly from Argentina, that were slightly resistant. A good representative of this group was T. I. 57. Under conditions of moderate attacks of blue mold in the plant bed, this genotype frequently showed striking evidences of resistance. However, when disease conditions were severe, resistance broke down almost completely. Subsequently, T. I. 57 was crossed with two flue-cured varieties, Gold Dollar and White Stem Orinoco, and large F_2 populations were grown and tested. Plants surviving severe attacks of blue mold were tested as F_3's. However, despite extensive work over a 3-year period, the full resistance of the T. I. 57 parent was never completely recovered. Evidently, resistance was dependent on a number of genes; consequently, it seems that there is little prospect of obtaining satisfactory resistance to blue mold in *N. tabacum* by intraspecific hybridization.

RESISTANCE OF VARIOUS SPECIES OF NICOTIANA

By means of the methods outlined, the comparative resistance of some 33 species of *Nicotiana* has been determined under what might

be termed plant-bed conditions as they prevail in the United States. This, as indicated, has involved tests in actual beds at the Coastal Plain Experiment Station, Tifton, Ga.; the Pee Dee Experiment Station, Florence, S. C.; the McCullers and Oxford branch stations, North Carolina; and the Arlington Experiment Farm, Arlington, Va.; and repeated tests in the greenhouse with transplanted seedlings 6 to 8 weeks old. Except for a number of species tested only a few times, the results are shown in table 2.

TABLE 2.—*Comparative resistance of Nicotiana species to blue mold under plant-bed conditions in the United States*

Group 0–1, immune to highly resistant	Group 2, moderately resistant	Group 3, slightly resistant	Group 4, susceptible
N. debneyi Domin.[1] N. exigua H.-M. Wheeler.[1] N. goodspeedii H.-M. Wheeler.[1] N. longiflora Cav. N. maritima H.-M. Wheeler.[1] N. megalosiphon Heurck and Muell.-Arg.[1] N. plumbaginifolia Viv. N. rotundifolia Lindl.[1]	N. acuminata (Grah.) Hook. N. caesia Suksd. N. gossei Domin.[1] N. paniculata L. N. rustica L.	N. alata Link and Otto. N. attenuata S. Wats. N. langsdorffii Weinm. N. otophora Griseb. N. quadrivalvis Pursh.[2] N. raimondii Macbride. N. repanda Lehm. N. sanderae W. Wats. N. sylvestris Speg. and Comes. N. tomentosa Ruiz and Pavon.	N. benthamiana Domin.[1] N. cavanillesii Dun. N. glauca Grah. N. glutinosa L. N. nesophila Johnst. N. nudicaulis S. Wats. N. stocktoni Brandeg. N. tabacum L. N. trigonophylla Dun. N. wigandioides Koch and Fint.

[1] Australian species; all other species are of American origin.
[2] This species has commonly been called *Nicotiana bigelovii* S. Wats.

For convenient consideration, the 33 species represented in table 2 have been divided into 4 groups on the basis of their resistance to blue mold, but actually they represented a complete gradation from highly susceptible to immune. The 8 species listed in group 0–1 showed no evidence of disease in any plant-bed test and hence might be listed as immune. Group 2 (moderately resistant) showed only a slight amount of disease under plant-bed conditions. Group 3 (slightly resistant) was a disease-tolerant group. The plants developed abundant infection but were rarely defoliated completely and usually recovered. Group 4 (susceptible) requires no comment other than to mention that *Nicotiana benthamiana*, *N. nesophila*, and *N. stocktoni* were more susceptible than the others and could have been designated as a separate "very susceptible" group.

As table 2 indicates, the Australian species on the whole were more resistant to blue mold than the American species. Of the 8 Australian species, 6 were highly resistant to immune; 1 was moderately resistant; and 1 (*Nicotiana benthamiana*) was highly susceptible. Wheeler (8)[5] lists *N. benthamiana* as native to the northwest coast of Australia, where blue mold presumably does not occur. Of the 25 American species, only 2 (*N. longiflora* and *N. plumbaginifolia*) have been rated as immune to highly resistant.

Reference has already been made to the effect of plant age on resistance to blue mold. Even plants of the susceptible species become more resistant as they grow older, and the plants of some species pass through a complete transformation from highly susceptible when very young to completely immune a few weeks later (fig. 2).

[5] Italic numbers in parentheses refer to Literature Cited, p. 87.

The resistance ratings in table 2 are based on severe plant-bed conditions and compare well with the reaction of greenhouse plants 6 to 8 weeks old. It is of much interest to know the reaction of younger plants of the species in the immune to highly resistant group of table 2. To obtain such data, seedlings were grown under uniform conditions and inoculated when they were 14, 24, and 35 days old. Since it was not feasible to transplant these very small plants, the seed was sown in pots and the plants were thinned to uniform stands. Experiments 1, 2, and 3, the data for which are shown in table 3, were made at widely separated intervals with three entirely separate crops of plants.

TABLE 3.—*Resistance to blue mold of Nicotiana plants 14, 24, and 35 days old*

Nicotiana species	Age of plants	Leaves sporulating Experiment No.—			Mean	*Nicotiana* species	Age of plants	Leaves sporulating Experiment No.—			Mean
		1	2	3				1	2	3	
	Days	*No.*	*No.*	*No.*	*No.*		*Days*	*No.*	*No.*	*No.*	*No*
N. tabacum	14	65	48	50	54.3	N. plumbaginifolia	14	38	15	11	21.3
	24	85	96	44	75.0		24	2	8	12	7.3
	35	130	104	42	92.0		35	0	8	2	3.3
N. glauca	14	50	54	48	50.7	N. debneyi	14	21	8	10	13.0
	24	76	52	67	65.0		24	0	8	5	4.3
	35	135	32	128	98.3		35	0	0	0	0
N. rustica var. humilis Schrank.	14	98	24	28	50.0	N. rotundifolia	14	0	14	7	7.0
	24	103	11	11	41.7		24	0	3	4	2.3
	35	60	8	24	30.7		35	0	0	0	0
N. rustica var. brasilia Schrank.	14	68	5	4	25.7	N. maritima	14	18	4	8	10.0
	24	6	11	0	5.7		24	0	3	14	5.7
	35	4	10	14	9.3		35	0	0	0	0
N. paniculata	14	60	23	43	42.0	N. megalosiphon	14	0	4	12	5.3
	24	42	44	6	30.7		24	0	3	0	1.0
	35	8	0	1	3.0		35	0	0	0	0
N. caesia	14	14	11	18	14.3	N. goodspeedii	14	2	4	0	2.0
	24	8	6	8	7.3		24	0	0	0	0
	35	6	11	8	8.3		35	0	0	0	0
N. longiflora	14	80	12	62	51.3	N. exigua	14	0	0	0	0
	24	24	12	34	23.3		24	0	0	0	0
	35	3	4	0	2.3		35	0	0	0	0

The species listed in table 3 include two that were rated as susceptible in table 2—*Nicotiana tabacum* and *N. glauca*; three that were moderately resistant—*N. rustica*, *N. paniculata*, and *N. caesia*; and all eight of the immune to highly resistant group. The amount of sporulation is the best available measure of plant resistance in tests of this sort; hence, the data in table 3 are given in terms of the number of sporulating leaves. It should be noted that in some species the actual number of leaves exposed was two or three times as many with plants 35 days old as with plants 14 days old. With a susceptible species such as *N. tabacum*, the increase in resistance as the plants grow is slow, and hence the larger number of leaves exposed with 35-day plants was usually reflected in a larger number of sporulating leaves. However, with the moderately resistant species *N. rustica* and *N. paniculata* this was not true. By the time these plants were 35 days old they had developed sufficient resistance to reduce greatly the actual number of sporulating leaves. *N. caesia* differed from any
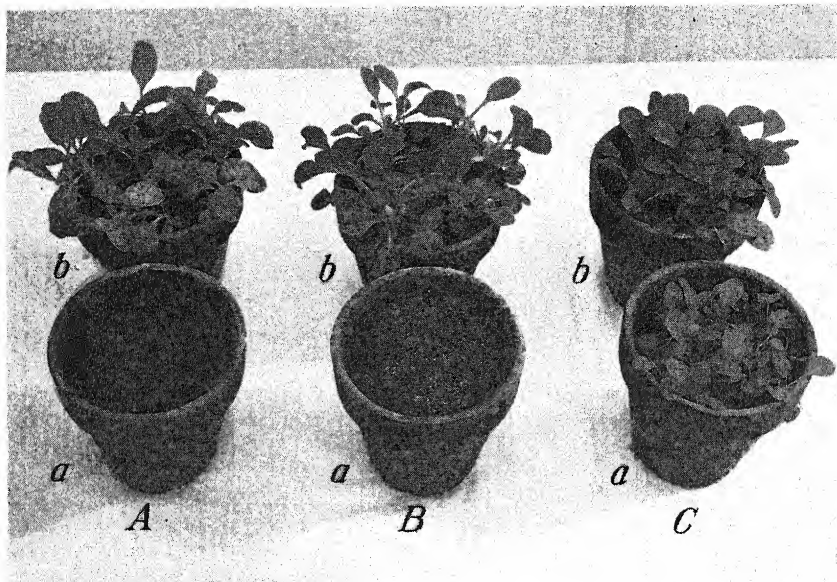
FIGURE 2.—Tobacco plants showing relation of age to susceptibility to blue mold. Each pot originally contained 25 plants; all were sown at the same time. *A, Nicotiana rustica: a,* Plants inoculated when 14 days old, susceptible and killed; *b,* plants inoculated when 35 days old, moderately resistant, disease limited to leaf lesions. *B, N. maritima: a,* Plants inoculated when 14 days old, completely susceptible; *b,* plants inoculated when 35 days old, immune. *C, N. exigua: a,* Plants inoculated when 14 days old; *b,* plants inoculated when 35 days old; both groups immune.

other species in that, while it was quite resistant at the start, resistance increased very little as the plants grew older.

Reference to table 3 shows that the difference between species such as *Nicotiana rustica, N. paniculata,* and *N. caesia,* which are in the moderately resistant group in table 2, and species such as *N. longiflora* and *N. plumbaginifolia,* which are in the immune to highly resistant group, is purely one of degree. As indicated earlier, resistant species showing no blue mold in any actual plant-bed test were placed in group 0–1 of table 2; but the results given in table 3 indicate that two of these, *N. longiflora* and *N. plumbaginifolia,* are really only a little more resistant than certain species classed as moderately resistant in table 2.

Nicotiana debneyi, N. rotundifolia, N. maritima, N. megalosiphon, N. goodspeedii, and *N. exigua,* as indicated in table 3, make a compact group of highly resistant species, although, if plants only 14 days old are inoculated, the first three of this group may show considerable leaf infection. To bring out this relation of plant age to susceptibility to blue mold still more clearly, an experiment was made in which the number of leaves exposed was counted so that data could be given on the basis of percentage of leaves sporulating. The percentage of plants killed was also noted (table 4).

TABLE 4.—*Relation of age of Nicotiana plants to their susceptibility to blue mold*

Nicotiana species	Age of plants	Leaves[1] sporulating	Plants [2] killed	Nicotiana species	Age of plants	Leaves[1] sporulating	Plants [2] killed
	Days	*Percent*	*Percent*		*Days*	*Percent*	*Percent*
N. tabacum var. Gold Dollar	15	100	100	N. longiflora	15	88	64
	25	100	100		25	30	0
	35	100	100		35	24	0
N. tabacum var. Maryland Broadleaf	15	97	96	N. plumbaginifolia	15	42	24
	25	100	100		25	2	0
	35	100	100		35	0	0
N. glauca	15	100	100	N. debneyi	15	32	28
	25	100	100		25	0	0
	35	100	100		35	0	0
N. alata	15	100	100	N. rotundifolia	15	0	0
	25	100	80		25	0	0
	35	100	76		35	0	0
N. rustica var. humilis	15	98	96	N. megalosiphon	15	0	0
	25	89	60		25	0	0
	35	60	0		35	0	0
N. rustica var. brasilia	15	75	44	N. exigua	15	0	0
	25	6	0		25	0	0
	35	3	0		35	0	0
N. paniculata	15	90	84				
	25	42	44				
	35	38	0				

[1] The number of leaves exposed averaged 100.
[2] The number of plants exposed averaged 25.

The data in table 4 show that in this experiment plants from 15 to 35 days old were equally susceptible in the species *Nicotiana tabacum* and *N. glauca* and equally resistant in the species *N. rotundifolia*, *N. megalosiphon*, and *N. exigua*; but in all species intermediate between these groups, susceptibility varied widely, depending on plant age. The results in table 4 suggest that *N. plumbaginifolia* was distinctly more resistant than *N. longiflora*; but, as indicated in table 3, the difference usually was not great, and both species reacted as highly resistant to immune by the time they were 6 to 7 weeks old. It is quite apparent, however, that anyone making resistance tests with very young *N. longiflora* seedlings would list this species as highly susceptible. *N. paniculata* and *N. rustica* also were very susceptible when young but usually became highly resistant by the time they were exposed to attack in the plant beds.

CROSSES BETWEEN NICOTIANA TABACUM AND RESISTANT SPECIES

Since the object of this work was to provide a foundation for the breeding of satisfactory blue mold resistant varieties of *Nicotiana tabacum*, the question arises as to whether *N. tabacum* can be successfully crossed with any of the group of highly resistant to immune *Nicotiana* species listed in table 2. The reports of Malloch and Malloch (*4*), Pal (*6*), Kostoff (*3*), and others are unanimous in concluding that self-fertile or cross-fertile hybrids have never been obtained between *N. tabacum* and any of these species. The recent development of colchicine naturally suggested its use, but extensive colchicine treatments on the seed and plants of these hybrids have given no results of positive value. Other methods, however, have gradually been developed that are more successful, and work is now in progress with progeny from *N. tabacum* crossed with *N. megalosiphon*, *N. debneyi*, *N. longiflora*, and *N. plumbaginifolia*. The results of this work suggest that it may be possible to use the very high degree of blue mold resistance found in certain wild *Nicotiana* species in breeding resistant va-
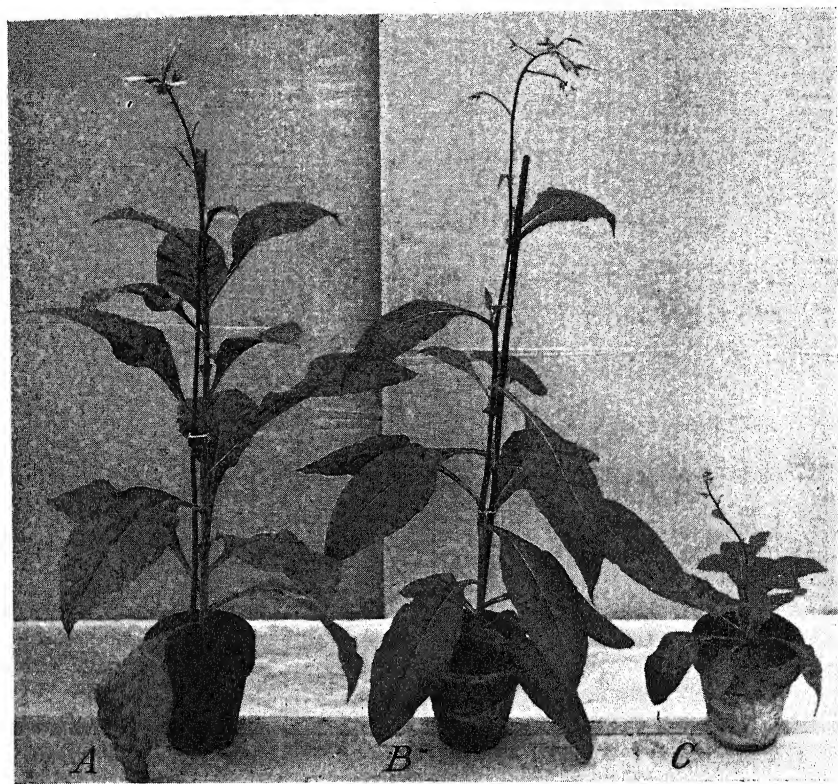
FIGURE 3.—Hybrid (*B*) produced by crossing blue mold resistant *Nicotiana debneyi* (*C*) with blue mold susceptible *N. tabacum* (*A*).

rieties of the cultivated *N. tabacum*. The undertaking is one of extreme difficulty, however, and the progress here reported represents merely the first step in its accomplishment (fig. 3).

DISCUSSION

Many investigators have reported briefly on the resistance of *Nicotiana* species to blue mold, but the reports are quite confusing. Thus, Angell and Hill (*2*) concluded that none of the *Nicotiana* species tested showed appreciable resistance to blue mold and hence that no promising basis for a program of breeding for disease resistance existed. On the other hand, Smith-White et al. (*7*) concluded that a high degree of blue mold resistance was available in the species, and they listed *Nicotiana debneyi* as immune. The writer has included *N. debneyi* among the group of species immune under plant-bed conditions in the United States (table 2). However, as indicated in tables 3 and 4, the 14-day-old seedlings of *N. debneyi* are quite susceptible to blue mold. Hence, depending on the age of the plants inoculated, *N. debneyi* might be reported as either susceptible or immune, and this situation obtains with other *Nicotiana* species. Therefore, unless information is available as to the age of the plants inoculated, data on susceptibility or resistance to blue mold are likely to

be misleading. In the reports on blue mold resistance already published, no mention has been made of this age factor; hence the basis for contradictory conclusions is evident.

In addition to age, there are other factors that tend to promote conflicting reports on blue mold resistance. One is the severity of disease attack. Certain *Nicotiana tabacum* genotypes may appear quite resistant under conditions of mild disease attack. The data in table 3 indicate the marked variability that can be expected in different tests. Host vigor has a marked effect on susceptibility. When slow-growing, tough tobacco plants and fast-growing, tender plants are equally exposed to blue mold, the former are scarcely affected, whereas the latter are defoliated. Another source of confusion has been the clouded taxonomic condition of the genus *Nicotiana*. Thus, Angell and Hill (*2*) referred to Adam (*1*) as finding that *N. longiflora* was susceptible to blue mold. However, inquiry reveals that the species tested by Adam was probably *N. suaveolens* var. *longiflora* Benth., which is now called *N. megalosiphon*. This is entirely different from the present *N. longiflora*, which is a South American species and does not occur in Australia.

A final source of variability in reports on species resistance, and one that may be of great importance, is the existence within a species of genotypes differing greatly in their resistance to blue mold. A distinct difference of this sort is shown in table 3 between *Nicotiana rustica* varieties *brasilia* and *humilis*. In these experiments, two collections of *N. longiflora* were used that were morphologically similar. However, this species is known to be very polymorphic, and Millán (*5*) listed four separate varieties differing greatly in appearance. Consequently it is quite possible that *N. longiflora* genotypes exist that differ greatly in their blue mold reaction from the ones tested. Therefore any conclusion as to species resistance can apply only to the particular material tested, and a complete picture could be secured only by testing not one or two but many collections of each species. The object of the present study was to establish a definite basis for a sound program of breeding for blue mold resistance. It is believed that this object has been achieved, because the resistance of any of the eight species making up group 0–1 of table 2 would be entirely adequate from the point of view of practical blue mold control in the United States.

SUMMARY

Tests with over 1,000 collections of *Nicotiana tabacum* have shown that none possessed resistance to blue mold adequate to provide a basis for a breeding program.

Tests with other species of *Nicotiana* have shown that many are highly resistant to blue mold but that the expression of resistance is complicated by a number of factors. The age of the plants tested was the most important single factor, and by exposing greenhouse-grown plants to blue mold when they were 6 to 8 weeks old results were obtained that compared closely with those obtained under the most severe out-of-door plant-bed conditions.

The age at which plants of different species became highly resistant or immune to blue mold was 6 to 7 weeks for *Nicotiana longiflora* and *N. plumbaginifolia;* 3 to 4 weeks for *N. debneyi, N. rotundifolia, N. maritima,* and *N. megalosiphon;* and 2 weeks for *N. goodspeedii. N.*

exigua was immune at all stages of growth. Under out-of-door plant-bed conditions no infection was observed on any of these eight species.

Successful crosses have been made between *Nicotiana tabacum* and *N. debneyi, N. megalosiphon, N. longiflora, and N. plumbaginifolia.*

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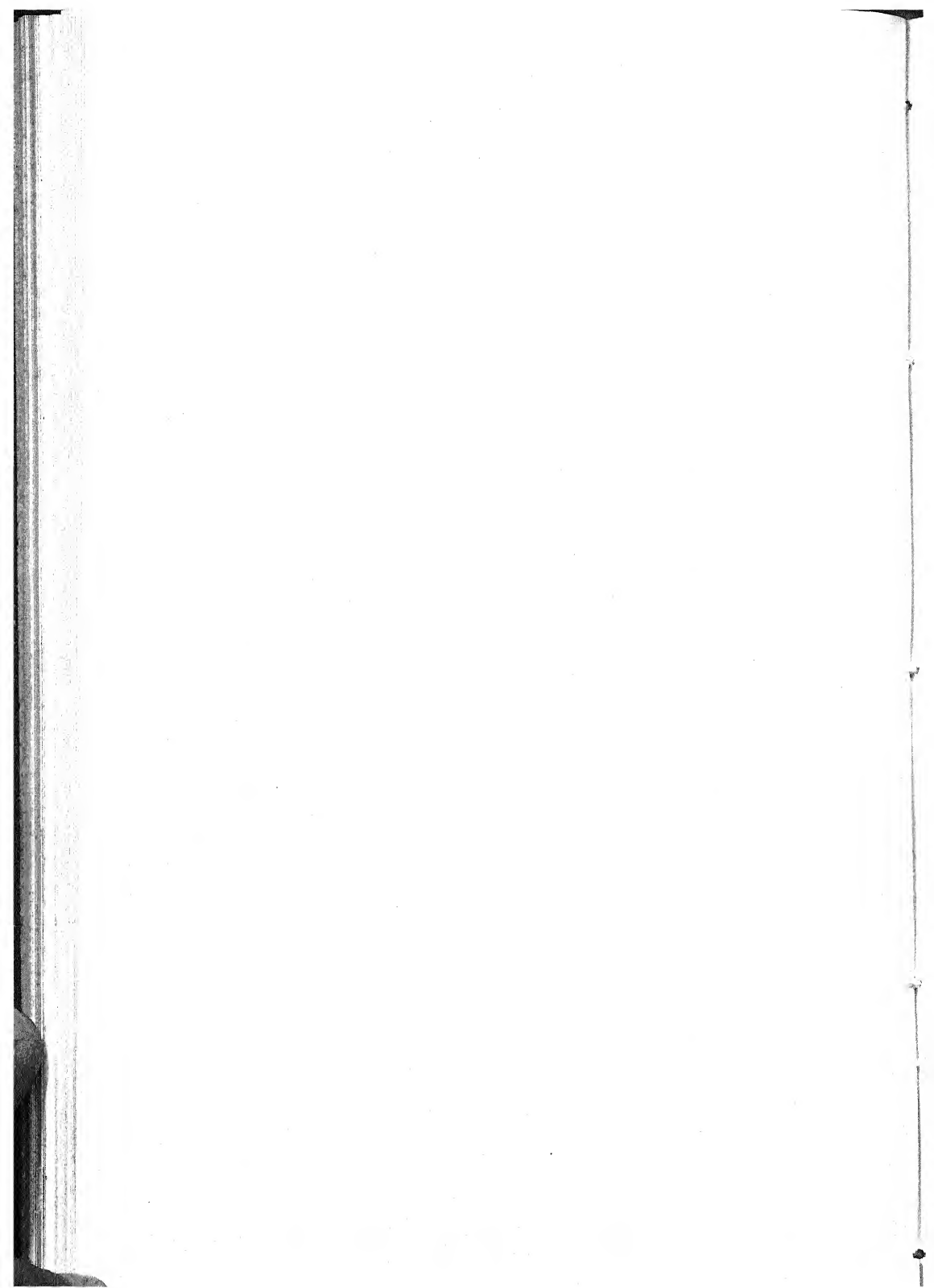
COMPOSITION AND DIGESTIBLE NUTRIENT CONTENT OF NAPIER GRASS LEAVES [1]

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INTRODUCTION

Napier grass (*Pennisetum purpureum* Schum.), also known as elephant grass and Merker grass, is a rank-growing perennial which is being used extensively for rotational pastures, as silage, and as a soil-improving crop, in Florida, Hawaii (*10, 11*),[3] Trinidad (*8, 9*), and other tropical and semitropical regions. This paper reports the results of a study to determine the composition and digestible nutrients of leaves of Napier grass harvested in a way to simulate grazing by cattle.

REVIEW OF LITERATURE

No analyses of Napier grass leaves or of that part of the plant consumed by cattle in natural grazing have been found in the literature. Wilsie, Akamine, and Takahashi (*10*) reported the composition of the "palatable portion" of the plant harvested at varying growth intervals which they designated as "all leaves and that part of the plant above and including the fifth visible ligule from the tip of the culm." Paterson (*8, 9*) reported the composition of two varieties, for the entire plant, harvested at short intervals.

Harrison (*4*) conducted digestion trials with fresh Napier grass and found the digestion coefficients to be: For crude protein 63, for crude fiber 64, for nitrogen-free extract 60, and for crude fat 57. The chemical composition and digestibility of Napier grass as reported in the literature are summarized in table 1.

The digestion coefficients for Napier grass silage were determined by Neal, Becker, and Arnold (*7*) to be: For crude protein 29, for crude-fiber 50, for nitrogen-free extract 40, and for crude fat 65. Digestibility experiments have been reported on Napier grass by Carbery, Chatterjee, and Talapatra (*2*), but as theirs was a study of mineral balances no digestion coefficients were included in the report.

EXPERIMENTAL METHODS

The digestion trial, carried out at Gainesville, Fla., was conducted according to the method outlined by Forbes and Grindley (*3*). The Napier grass consisted of the leaves with the tender-growth tips plucked by hand twice daily in a manner to simulate grazing, from plots representing the growth of the preceding 4 weeks, the plots having been harvested in the same manner at that time. This freshly cut Napier grass constituted the entire nutrient intake of four mature Jersey steers for 4 consecutive 5-day experimental periods, following a 10-day preliminary feeding period. The feces were collected manually and stored in galvanized-iron cans. Samples were taken daily for dry-matter analyses and in triplicate for nitrogen determinations on the fresh material. Dry-matter samples were composited by 5-day periods for proximate analyses.

TABLE 1.—*Composition and digestibility of Napier grass as reported by several investigators*

WILSIE, AKAMINE, AND TAKAHASHI (*10*)

Growth period (weeks)	Digestion trial No.	Composition on dry basis						Total digestible nutrients
		Dry matter	Crude protein	Crude fiber	Nitrogen free extract	Crude fat	Ash	
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
6			7.9	28.8	41.9	2.2	19.2	
8			6.9	30.7	41.2	2.0	19.2	
10			6.0	32.0	42.5	1.9	17.6	
12			5.9	32.6	42.4	2.0	17.1	
14			5.6	33.9	43.1	2.0	15.4	

PATERSON (*8*)

4		14.5	9.9	24.0	[1] 47.3		18.8	
8		16.5	7.4	27.4	[1] 48.4		16.8	
12		20.7	6.1	31.5	[1] 45.7		16.7	

PATERSON (*9*)

2		13.3	11.7					
4		13.7	9.8					
6		13.3	7.5					
2		12.2	11.9					
4		12.8	9.2					
6		14.8	7.5					

HARRISON (*4*), ON FRESH BASIS [2]

	I	16.7	1.3	4.9	7.8	0.3	2.4	
	II	17.3	1.9	5.1	8.4	.2		
	III	19.8	1.5	6.9	8.3	.4	2.7	

HARRISON (*4*), DIGESTION COEFFICIENTS

	I		64.0	62.0	61.0	45.0		
	II		65.0	58.9	54.2	66.6		
	III		60.2	71.6	64.2	60.2		

HARRISON (*4*), DIGESTIBLE NUTRIENTS

	I		0.8	3.0	4.8	0.1		8.7
	II		1.2	3.0	4.6	.1		9.0
	III		.9	4.9	5.3	.2		11.3

[1] Includes crude fat.
[2] Harrison presented original data on fresh basis.

In separate investigations at the Florida Agricultural Experiment Station by Blaser, Kirk, and Arnold to evaluate Napier grass under rotational grazing with beef and dairy cattle, samples were plucked at regular intervals from protected quadrats in a manner to simulate grazing in the remainder of the field. These quadrats were moved at regular intervals. The harvested material was used to represent the composition of the grass as grazed. Proximate analyses were made by methods of the Association of Official Agricultural Chemists (*1*). Calcium, magnesium, and phosphorus were determined by the method of Morris, Nelson, and Palmer (*5*).

RESULTS OF EXPERIMENTS

The composition of the Napier grass as used in the digestion trials, and from the separate grazing tests conducted by Blaser, Kirk, and Arnold for a 2-year period, are presented in table 2. The ranges among samples used to compute the average composition were:

TABLE 2.—*Composition, coefficients of digestibility of nutrients, and digestible nutrient content of Napier grass leaves harvested in a manner to simulate grazing*

COMPOSITION OF FRESH GRASS

Source of Samples	Dry matter	Crude protein	Crude fiber	Nitrogen free extract	Crude fat	Ash	Total digestible nutrients
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Digestion trials [1]	20.8	2.7	6.0	9.4	0.9	1.8	
Grazing trials [2]	21.4	2.8	6.5	9.8	0.7	1.6	

COEFFICIENTS OF DIGESTIBILITY

Steer:							
E 58	64.6	65.0	66.3	68.8	56.0		
E 69	66.6	65.1	70.1	68.8	59.2		
E 71	66.0	65.2	65.2	70.1	57.2		
E 76	66.5	64.2	69.5	70.4	58.1		
Average	66.0	65.0	68.0	70.0	58.0		

DIGESTIBLE NUTRIENTS

Digestion trials [1]		1.8	4.1	6.6	0.5		13.7
Grazing trials [2]		1.8	4.4	6.9	.4		14.0

[1] Digestion trial records are based on analyses of 21 samples taken Aug. 11 to 30, 1938.
[2] 23 samples of Napier grass represent 2 entire seasons for 1 area, and 1 season for a second area under rotational grazing; unpublished data used by permission of Blaser, Kirk, and Arnold.

Dry matter 16.5 to 24.0 percent, crude protein 9.9 to 17.5 percent, crude fiber 26.5 to 33.6 percent, crude fat 2.4 to 4.5 percent, and ash 6.0 to 8.4 percent. The ash content is less than half that of the values given in table 1. The average content of calcium, of magnesium, and of phosphorus were respectively, 0.50, 0.18, and 0.35 percent. The dry matter and protein content of the leaves is higher than reported by Wilsie, Akamine, and Takahashi (*10*), Paterson (*8, 9*), or Harrison (*4*), indicating that the materials are not altogether comparable.

Each of the four steers consumed approximately 15.5 pounds of dry matter daily, or about 75 pounds of fresh grass, which was sufficient to maintain their weight or to cause slight gains. The daily

feed intake and fecal output of the steers were quite uniform throughout the trial. The average digestion coefficients for the respective steers are presented in table 2.

The values for digestible nutrient content presented in table 2 were found by applying the digestion coefficients to the analyses in the same table. The fresh grass, having a dry matter content of 21.4 percent, averaged 1.8 percent digestible crude protein and 14.0 percent total digestible nutrients, or 8.5 and 65.7 percent respectively on a moisture-free basis.

The digestibility of fresh Napier grass and other comparable forages is presented in table 3. Napier grass leaves as grazed were more digestible than the whole plant as determined by Harrison but were not as digestible as mixed pastures grasses and clovers from closely grazed fertile pasture. However, they compared favorably with mixed immature grasses; were more digestible than bluegrass, redtop or timothy, and much more digestible than the more mature Napier grass plant after ensiling.

TABLE 3.—*Digestion coefficients of the nutrients in Napier grass as compared with those in other pasture grasses reported by Morrison (6, Appendix, table 1)* [1]

Forage	Number of trials	Crude protein	Crude Fiber	Nitrogen-free extract	Crude fat
Napier grass leaves [2]	4	65	68	70	58
Napier grass [3]	3	63	64	60	57
Native bluegrasses	2	64	45	60	50
Grasses, mixed, immature	2	70	66	75	62
Orchard grass	3	60	60	55	54
Pasture grasses and clovers, mixed, from closely-grazed, fertile pasture	51	77	76	78	56
Redtop	3	61	61	62	50
Sudan grass	4	72	76	69	72
Timothy	3	48	56	66	53

[1] Data used by permission of the author.
[2] From table 2 of this paper.
[3] Harrison (4).

SUMMARY

Napier grass leaves as grazed rotationally by cattle provide pasture which is comparable in nutrient content with other pasture grasses. The digestion coefficients were found to be: For crude protein 65, for crude fiber 68, for nitrogen-free extract 70, and for crude fat 58, as compared with average coefficients of 63, 64, 60, and 57, respectively, as determined by Harrison. The fresh grass provided 1.8 percent of digestible crude protein and 14 percent of total digestible nutrients, or 8.5 and 65.7 percent respectively, on a moisture-free basis.

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STRAWBERRY BREEDING STUDIES INVOLVING CROSSES BETWEEN THE CULTIVATED VARIETIES (× FRAGARIA ANANASSA) AND THE NATIVE ROCKY MOUNTAIN STRAWBERRY (F. OVALIS) [1]

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INTRODUCTION

The strawberry is perhaps the most widely grown of all the fruits in the United States; it is not only particularly adapted to home gardens but is also a highly important fruit of commerce. However the strawberry plant is too tender to survive and produce satisfactorily without winter protection in almost all of the northern half of the United States, especially in the northern Great Plains areas where winters are very severe. Consequently, the development of hardier varieties should reduce the cost of growing strawberries where winter protection is now necessary.

The value of the native Rocky Mountain strawberry (*Fragaria ovalis* (Lehm.) Rydb.) in breeding more desirable varieties of cultivated strawberries (× *F. ananassa* Duch.) has been pointed out by Georgeson (7),[3] Darrow (2), and Hildreth and Powers (9). It should be noted, however, that these workers used the species names *platypetala* and *cuneifolia* instead of *ovalis*. Crosses between cultivated varieties and collections of *F. ovalis* have been grown at the United States Department of Agriculture Cheyenne Horticultural Field Station, Cheyenne, Wyo., for a number of years. This paper reports the progress being made there in breeding strawberries, especially that dealing with characters of economic importance, and it evaluates the breeding methods used. The immediate objective of the breeding program is to recombine the large size of fruit of the commercial varieties with the winter hardiness of the native Rocky Mountain strawberry.

REVIEW OF LITERATURE

The literature dealing with strawberry breeding is rather extensive, and only that part having a direct bearing on the present study will be reviewed here. The reader is referred to Bunyard (1) for a more complete discussion of the history and development of the strawberry and to Darrow (2) for a review of the literature dealing with breeding.

Bunyard (1) pointed out that even though the wild strawberries, including *Fragaria virginiana*, had been grown as garden plants for some 300 years before the introduction of *F. chiloensis* into France by

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Frezier, no improved variety had been recorded. His following remarks are particularly interesting:

> We see, therefore, that *F. virginiana*, left merely to the stimulus of cultivation, did not produce any varieties of special size or remarkable in other qualities, and it was generally admitted that for flavour the original type was unsurpassed. Nothing at all approaching in size the Strawberry of modern times had been produced, and the reason of this was, doubtless, that no character giving large fruit had been introduced into the gametic constitution of existing fruits.
>
> The introduction of the Chilian Strawberry, *F. chiloensis*, brought, however, the required size into combination with the flavour of the Virginian, and thus laid the foundation of the fruit as we know it to-day. The introduction of the Chilian Strawberry was, therefore, an event of the first importance in Strawberry history . . .

Hence, it seems that the modern cultivated strawberry resulted from crosses involving *F. virginiana* and *F. chiloensis*. This conclusion is in accord with that of Darrow (*2*).

The literature dealing with the use of *Fragaria ovalis* to improve the cultivated strawberry is limited. Georgeson (*7*), working in Alaska, used *F. chiloensis, F. ovalis*, and the cultivated strawberry to develop a berry suitable for Alaskan conditions. He succeeded in producing strawberries suitable for the interior of Alaska, where the temperature often falls as low as 50° F. below zero and sometimes as low as 65° below. The following statement by Georgeson (*7, p. 8*) sets forth the merits of one of his hybrids:

> One of the best hybrids so far produced has resulted from crossing the Magoon, a well-known strawberry of the Pacific Coast, with pollen from the wild plant of the interior. This hybrid, so far as tested, possesses all the desired qualities. The berry is large, of deep red color not merely on the surface but throughout, and it is firm enough to stand shipment. The berry has a good flavor and a calyx that separates easily when the fruit is ripe.

Darrow (*2*) pointed out the value of *Fragaria ovalis* as a source of cold and drought resistance, everbearing types, and earliness.

Hildreth and Powers (*9*) reported the results from tests involving extensive collections of the Rocky Mountain strawberry. In 1936 systematic collections were made under the supervision of A. C. Hildreth. About 42,000 plants were collected from more than 1,100 localities in Wyoming, Colorado, Montana, New Mexico, and Utah. None of these had fruits large enough for commercial production, although they were generally of high quality. As grown in the trial plots, these native plants show variation in many horticultural characters, differing in size, shape, color, and quality of fruit and in season of maturity, fruiting habit, prolificacy, tendency to produce runners, resistance to disease, and tolerance to soil alkalinity. Some are distinctly everbearing, others are June bearers. Some show considerable drought resistance, and the majority of them have proved hardy without protection at Cheyenne, Wyo. The F_1 plants from crosses between cultivated varieties and different collections of the native strawberry were found to vary within and between crosses as regards the following horticultural characteristics: Size, sweetness, flavor, aroma, shape, color, prolificacy, and time of maturity of fruit; June or everbearing habit; position of fruits on the plant; type of flower cluster; shape, size, and abundance of leaves; and habit of plant growth.

Another problem in the breeding of *Fragaria* arises from the fact that some supposedly F_1 hybrids resemble the maternal parent and

others resemble the paternal parent. Such cases have been reported by Millardet (*11*), East (*4, 5*), Ichijima (*10*), and Rygg and Darrow (*15*). Ichijima described both maternal and paternal types of inheritance. In the case of maternal inheritance the chromosome number was that of the female parent, and in case of paternal, that of the male parent. All these populations of matroclinous type show no trace of hybridity in their genoms. On the other hand, the hybrid nature of the genoms never failed to show in those populations that exhibit their hybrid character morphologically. The exact method of occurrence of these unusual types is not known, but it is evident that among the F_1 populations there occur individuals which possess the nuclear complex of one or the other parent. The possibility of the occurrence of these exceptional individuals must be taken into account in breeding or genetic studies involving *Fragaria*.

EXPERIMENTAL CONSIDERATIONS

MATERIAL

Since the mode of reproduction in the cultivated strawberry is asexual, only one plant combining the desired characters in any segregating population is necessary for the production of a variety possessing the traits sought. Most strawberry varieties have perfect flowers, but a few have pistillate flowers only. Varieties having perfect flowers may be cross-pollinated by insects, and in hybridizing strawberries precautions must be taken to prevent off pollinations.

The results of a study of the behavior of the chromosomes during meiosis in the parents and in the crosses between cultivated varieties and the Rocky Mountain strawberry have already been reported (*14*). Chromosome behavior during meiosis of the parents and F_1 hybrids was found to be essentially normal. However, some irregularities similar to those found during meiosis of *Triticum* (*12*) do occur.

Because of a lack of sufficient breeding, genetic, and karyological information for crosses involving the cultivated varieties and the native Rocky Mountain strawberry, selection of the parents and the methods of breeding were based upon the phenotypic characters of the varieties and collections. The variety Gem was used in crosses because of its large fruit under the environmental conditions prevailing at Cheyenne and its everbearing habit, and because it comes nearer to being winter hardy than any of the other cultivated varieties tested. However, the fruit of Gem is lacking in quality. Of the commercial varieties of high quality tested by Hildreth at the Cheyenne Horticultural Field Station, Fairfax and Dorsett came the nearest to being winter hardy. Therefore, they were selected along with Gem as the cultivated varieties to be used in the strawberry-breeding program.

When collections of the Rocky Mountain strawberry were being selected for crossing, winter hardiness was given the first consideration. Since most of the collections of *Fragaria ovalis* possessed this trait (*9*), selection for other desirable characteristics was made easier. Collections as genetically diverse as possible were sought. Without the aid of intercrosses and progeny tests to determine genetic disparity, phenotypic differences were used for making the selections. The collections having winter hardiness and meeting these requirements were 36979, 361477, and 37501. In addition, 36979 had high quality

and was very prolific and 37501 bore larger fruit than the average collection of the Rocky Mountain strawberry and also had fair quality.

In analyzing and interpreting the experimental data it should be kept in mind that all of these parents may be heterozygous for at least some characters and that all plants of a certain collection are not necessarily alike genetically.

EXPERIMENTAL DESIGN

The experiment consisted of two parts: That dealing with breeding methods based upon fundamental genetic principles and that dealing with methods of conducting the experiment based primarily upon the fundamentals of biometry.

Methods of breeding in which hybridization is utilized may be broadly classified into two categories. The first is inbreeding followed by hybridization, which is particularly suited for breeding crops that are not self-pollinated and in which heterosis of some characters is of economic importance. It is employed when the available material is both highly heterozygous and heterogeneous and does not contain lines, strains, varieties, or species possessing sufficient genetic disparity for heterosis in the crosses to be of economic importance. The second is hybridization before inbreeding, which is used with crops in which lines, strains, varieties, or species possessing distinct genetic disparity do exist.

Since the commercial varieties of strawberries possess large fruits and are not winter hardy as compared with the native Rocky Mountain strawberry, and the latter possesses small fruits and as a rule is extremely winter hardy, it would seem that the primary objective of the breeding program would be attained most easily and rapidly by hybridizing before inbreeding.

Most F_1 hybrids originating from parents as diverse phenotypically as the cultivated varieties of strawberries and the native Rocky Mountain strawberry show decided heterosis of the vegetative parts of the plant. The problem, therefore, was to find a method of handling the hybrid material that would allow segregation of the genes differentiating size of fruit and winter hardiness and still maintain heterosis of the vegetative parts. The solution was found in the use of double crosses. For a better understanding of the problem the parental material is grouped below on the basis of the characters that are to be given primary consideration:

> Non-winter-hardy varieties with large fruit ($\times$*Fragaria ananassa*): Fairfax, Dorsett, Gem.
> Winter-hardy collections with small fruit (*F. ovalis*): 36979, 37501, 361477.

If a stands for the cultivated varieties and o for the collections of *Fragaria ovalis*, the possible ways of making the double crosses are as follows: $F_1(a \times a) \times F_1(o \times o)$ and $F_1(a \times o) \times F_1(a \times o)$. If the double cross is made in the manner first indicated, there will be a minimum of segregation of the genes differentiating degrees of winter hardiness, size of fruit, and vegetative vigor, since the greatest genetic disparity as judged by the differences between phenotypes would be expected to occur between the two groups a and o. However, if the cross is made according to the second method there will be a maximum of segregation of the genes differentiating winter hardiness, size of fruit, and vegetative vigor. This is just what is desired, as it provides

greater possibilities for obtaining plants combining winter hardiness and large size of fruit. However, such is not the case with vegetative vigor because, if the original F_1 plants exhibited heterosis and if the genetic disparity between the commercial varieties and that between the collections of the Rocky Mountain strawberry were negligible, then the double crosses made in the second manner should show a decided decrease in heterosis or vegetative vigor of plants. The double crosses actually made were $F_1(a \times o) \times F_1(a \times o)$, because this allowed a maximum segregation of the genes differentiating size of fruit and winter hardiness.

The design of the experiment was similar to that described in an earlier paper (*13*), and since the reasons for including the different populations have already been given, they need not be repeated here. The experimental design was a randomized complete block for the seed (achene) germination and the field studies. The number of blocks for the field studies was 10 and the number of plants per plot was 12. Since an inadequate number of plants was obtained for the F_1 population of Fairfax $\times$ 37501 and the selfed progeny of Fairfax, these populations were included in the first 6 replications only. In all comparisons and deductions including these populations, this fact was taken into account.

The symbol D. C. is used to designate a double cross involving two F_1 hybrids; S_1 is used to designate the progeny obtained by self-pollinating the parent indicated; and B_1 is used to designate the progeny obtained by backcrossing the F_1 hybrid Fairfax $\times$ 36979 to the parent indicated.

The populations in the breeding program, together with the number of plots grown per block for the field studies, are as follows:

Population:	Plots per block (number)
Fragaria ovalis:	
37501 (asexual)	1
361477 (asexual)	1
36979 (asexual)	1
36979 (S_1)	1
Hybrid populations:	
$B_1[F_1(\text{Fairfax} \times 36979) \times 36979]$	1
$F_1(\text{Fairfax} \times 36979)$	1
$F_1(\text{Fairfax} \times 361477)$	1
$F_1(\text{Fairfax} \times 37501)$	1
$F_2(\text{Fairfax} \times 36979)$	3
D. C. $[F_1(\text{Fairfax} \times 36979) \times F_1(\text{Dorsett} \times 37501)]$	4
D. C. $[F_1(\text{Fairfax} \times 36979) \times F_1(\text{Gem} \times 361477)]$	3
$B_1[\text{Fairfax} \times F_1(\text{Fairfax} \times 36979)]$	3
$\times$ *F. ananassa:*	
Fairfax (S_1)	1
Fairfax (asexual)	1
Gem (asexual)	1
Dorsett (asexual)	1

The original purpose of the study of seed germination was to determine whether there were differences in percentage and time of germination between populations and within populations and the relative importance of possible differences. As the investigation progressed an attempt was also made to determine the effect of the female parent upon the percentage and time of germination.

Since another objective was added after the experiment was started, the investigations consisted of the 2 rather distinct parts just men-

tioned. As regards the first part of the germination studies, the variates were the same as those for the field studies except that, of necessity, those asexually propagated were omitted and the number of plots per block differed for some of the populations. These differences were as follows. There were 4 plots per block of the F_2 population, 5 per block of the double cross involving Dorsett, and 5 per block of the backcross to Fairfax. The number of blocks for these seed germination studies was 17 and the number of seeds per plot was 25, whereas the number of blocks for the second germination study was 20 and the number of seeds per population was 500. The seeds were placed between moist blotters in the germinator on February 12, 1940, and August 22, 1941, respectively, and held for 140 days at an average temperature of 30° C. The seeds were approximately 1 year and 2½ years old, respectively. The greatest range in temperature was from 28° to 32°. The young plants were kept in the blotters until they developed roots and cotyledons and then were transferred to 2-inch pots. This allowed the recording of abnormal seedlings. Since such seedlings were so rare as to be negligible in these germination studies, they will not be considered further. The number of germinated seeds was recorded daily. However, in reducing the data they were classified into 5-day periods. For example, the first period for the seeds put in the germinators on February 12, 1940, is 40 (see table 1), and the frequencies given represent the number of seeds which germinated 38 to 42 days after the seeds were placed in the germinators.

CHARACTERS

In studying biological characters it is desirable whenever possible to record the results in standard units. However, because of the nature of the character or the time and labor involved in employing standard units, it is sometimes necessary to resort to observation. In the present work it was possible to measure the following characters by standard units: Germination (seeds germinated), plant height (centimeters), length of period from May 1 to first bloom (days), length of period from first bloom to first fruit ripe (days), and length of period from May 1 to first fruit ripe (days).

For all characters for which grades were used the numerical values were 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 5.5. To establish these grades the range in expression of the character for the parents and F_1 hybrids was first determined. Then tentative grades of 1, 2, 3, 4, and 5 were set up and these in turn were used to establish the fractional grades. With these tentative grades in mind the classification of the experimental material was started. Usually after the classification of two or three replicates any necessary adjustment of grades had been made and the grades had become fixed in mind. Then the classification was made of all replicates. During the classification of some characters it was found that the work was expedited by having samples of the middle grades (1.5, 3.0, and 4.5) available for making comparisons.

The notes on winter hardiness were taken on May 8, 1941, and were checked on June 3. All the plants in a hill (plants derived from one original plant by asexual propagation) receiving a grade of 0.5 were entirely free from any discernible injury. A grade of 1.5 was given to hills that had a few plants showing slight injury. Grades from 3.0 to 5.5, inclusive, were given to hills in which all the plants showed some

crown injury. Grades of 4.5 and above were given to hills the plants of which looked as if they would not survive even though some growth had started on May 8. By June 3 all the plants of most of those receiving grades of 4.5 or higher were dead. Hills having all plants dead on May 8 were given a grade of 5.5. The grades not specifically mentioned represent degrees of winter injury falling between those discussed.

Grades for size of fruit were established as follows. After the F_1 hybrids and parental varieties of the first two replicates had been examined, fruits having the following greatest diameters were taken as the standard for the grades designated:

Grade:	Diameter (inches)
1	$\frac{5}{16}$
2	$\frac{8}{16}$
3	$1\frac{2}{16}$
4	1.0
5	$1\frac{1}{4}$

The fractional grades, with the exception of 0.5 and 5.5, fell between the indicated measurements. The value 0.5 was assigned to all fruits smaller than grade 1 and 5.5 to all fruits larger than grade 5.0.

The plants per hill were counted for the first 5 replicates to determine the grades for runner plants. Then, on September 19 and 20, 1940, the plants of each replicate were classified. As these notes were taken before the hills derived from a single plant had been trimmed to occupy a 15- by 15-inch space, the grades would be expected to record the ability of the seedling or asexually propagated plant to produce runners. To determine whether such actually was the case, a correlation coefficient which involved the grades for each plant of the first 5 replicates and the number of plants per hill was calculated. The correlation coefficient was $+0.853$, which shows that the grades are a reliable estimate of the ability of the original plants to produce runners. By calculating the regression equation, it was found that the number of plants per hill increased 15.2 for each increase of 1 grade.

Sweetness, flavor, and firmness were ascertained by tasting fruits of each plant. The grades for flavor were based on the intensity of the strawberry flavor relished by most people. During the taking of this note it was observed that the intensity of flavor seemed to be positively associated with the higher degrees of sweetness.

EXPERIMENTAL RESULTS

The experimental results are divided into two parts, one dealing with the germination studies and the other with the characters taken in the field.

GERMINATION STUDIES

The data on amount and time of germination are given in table 1, and the χ^2 values for testing the statistical significance of the differences between populations as regards both the ratio between germinated and nongerminated seeds and the time of germination are given in table 2. These values are the heterogeneity χ^2's. The method of partitioning the data and the formulas used were given in an earlier paper (*14*) and need not be repeated here. In calculating the χ^2 values the data were grouped so that the least number of individuals expected in any one category was five or more (see Fisher *6*).

TABLE 1.—*Frequency distributions of germinating strawberry seeds approximately 1 year old*

Population	Seeds germinating after being in the germinator for the indicated number of days																					Total germinating
	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	135	140	No.
Fragaria ovalis (36979), S_1	25	124	97	32	12	5	1	2	0	2	0	0	0	0	0	1	0	0	1	0	0	304
B_1 [F_1 (Fairfax × 36979) × 36979]	158	121	47	21	6	1	18	1	1	2	0	0	1	0	0	0	1	0	1	0	1	372
F_1 (Fairfax × 36979)	3	19	26	30	19	24	18	5	9	4	4	3	5	5	5	3	1	3	1	1	0	185
F_1 (Fairfax × 361477)	0	7	32	58	27	19	14	10	11	9	9	5	5	7	1	8	1	3	3	1	2	240
F_1 (Fairfax × 37501)	3	17	40	35	17	19	9	2	7	8	1	13	2	9	1	4	2	3	4	1	1	198
Subtotal	6	43	98	123	63	67	41	17	27	21	14	21	12	21	7	15	4	10	6	3	4	623
Subtotal grouped[1]		49	98	123	63	67	41	17	27	21	35		33		28	31	19	25		29	23	623
F_2 (Fairfax × 36979)	134	151	45	7	7	5	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	352
F_2 (Fairfax × 36979)	79	100	59	19	8	5	0	2	0	2	1	1	0	0	1	0	0	0	0	0	0	277
F_2 (Fairfax × 36979)	126	145	52	14	5	6	3	1	0	1	0	0	0	0	0	0	0	0	0	0	0	353
F_2 (Fairfax × 36979)	66	152	102	25	11	9	1	2	1	1	1	0	1	0	1	1	0	0	0	0	0	375
Subtotal	405	548	258	65	31	25	4	6	1	4	2	2	1	1	2	1	0	1	0	0	0	1,357
Subtotal grouped[1]	405	548	258	65	31	25												25				1,357
D.C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	316	85	8	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	413
D.C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	204	164	30	9	0	0	2	0	1	0	0	0	1	0	0	0	0	0	0	0	0	405
D.C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	291	94	14	3	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	410
D.C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	226	159	22	2	0	0	2	0	0	1	0	0	0	0	0	1	0	0	0	0	0	411
D.C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	232	151	16	1	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	402
Subtotal	1,269	653	90	19	2	0	4	1	1	0	0	0	1	0	0	1	1	0	0	1	0	2,041
Subtotal grouped[1]	1,269	653	90	19	2		4		1				1				1					2,041
D.C. [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)]	58	189	104	21	8	4	3	2	0	0	0	0	0	0	0	0	1	1	0	0	0	396
D.C. [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)]	43	164	106	30	13	4	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	363
D.C. [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)]	56	156	94	23	12	4	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	246
Subtotal	157	509	304	79	33	9	6	4	2	0	0	0	0	0	0	0	1	1	0	0	0	1,105
Subtotal grouped[1]	157	509	304	79	33	9	6	4	2								1	23				1,105
B_1 [Fairfax × F_1 (Fairfax × 36979)]	2	12	45	41	18	43	14	12	14	4	5	8	6	4	3	0	3	0	0	0	0	231
B_1 [Fairfax × F_1 (Fairfax × 36979)]	11	22	50	53	30	45	6	14	12	9	7	4	4	1	4	4	2	0	1	2	1	278
B_1 [Fairfax × F_1 (Fairfax × 36979)]	4	10	67	39	20	22	11	6	7	8	5	3	4	7	5	6	4	4	2	5	2	238
B_1 [Fairfax × F_1 (Fairfax × 36979)]	17	48	56	37	18	32	14	8	5	10	10	6	4	2	5	0	1	0	0	0	0	275
B_1 [Fairfax × F_1 (Fairfax × 36979)]	10	58	40	26	28	32	14	9	6	7	7	7	3	7	5	4	1	2	2	0	2	237
Subtotal	44	150	258	196	114	150	52	49	44	40	34	28	21	21	21	10	9	6	3	7	5	1,262
Subtotal grouped[1]	44	150	258	196	114	150	52	49	44	40	34	28	42		21	31					30	1,262
× *F. ananassa* (Fairfax), S_1	3	7	12	20	21	17	6	8	11	7	3	1	6	2	3	1	0	2	1	1	0	132
Theoretical	37.9	39.5	21.6	10.2	5.2	5.0	2.1	1.6	1.6	1.4	1.0	1.0	0.8	0.8	0.6	0.5	0.3	0.2	0.2	0.2	0.2	132
Theoretical grouped	37.9	39.5	21.6	10.2	5.2	5.0	1.6	1.6	1.6	6.7	1.0	1.0	0.8		0.6		0.3				5.9	132
Grand total	2,067	2,155	1,177	555	282	274	116	87	87	74	53	52	42	46	33	28	14	20	12	12	10	7,196

[1] Each subtotal grouped includes the totals in the preceding line that are not included in the immediately preceding subtotal grouped.

For example, to test whether differences existed between populations as regards time of germination, the theoretical for the populations (in this case selfed Fairfax, table 1) having the smallest total number was calculated and the data were grouped accordingly. Grouping was also necessary for testing whether the differences noted within populations as to time of germination were statistically significant. The groupings of table 1 account for the fact that the total degrees of freedom listed for time of germination (table 2) are the sum of the degrees of freedom for grouped data (105), for between means of populations (49), and for periods (7).

TABLE 2.—x^2 *values for testing the statistical significance of the differences noted in table 1 between populations as regards the ratio between germinated and non-germinated seeds and the time of germination*

Variation due to—	Degrees of freedom for grouped data	Degrees of freedom	x^2
Total		482	
Populations		22	
Periods		20	
Grouping on the basis of the theoretical number of seeds in each category		183	
Ratio between germinated and nongerminated seeds		22	
Between means of populations		7	1,759.333
Within populations		15	146.453
F_1 (Fairfax×36979)		2	15.563
F_2 (Fairfax×36979)		3	80.422
F_1 (Fairfax×36979)×F_1 (Dorsett×37501)		4	[1] 5.131
F_1 (Fairfax×36979)×F_1 (Gem×361477)		2	26.321
B_1 [Fairfax×F_1 (Fairfax×36979)]		4	19.016
Time of germination		[2] 161	
Periods		7	
Between means of populations		49	4,715.474
Within populations		118	409.637
F_1 (Fairfax×36979)	14	24	38.551
F_2 (Fairfax×36979)	21	18	76.702
F_1 (Fairfax×36979)×F_1 (Dorsett×37501)	28	12	101.913
F_1 (Fairfax×36979)×F_1 (Gem×361477)	14	10	[1] 7.934
B_1 [Fairfax×F_1 (Fairfax×36979)]	28	54	184.537

[1] Odds against the deviations noted being due to chance are less than 19 to 1 ($P>0.05$).
[2] Sum of degrees of freedom for periods, for between means of populations, and for grouped subtotals.

The percentages of the seeds that germinated follow:

	Germination (percent)
Population:	
D. C. [F_1 (Fairfax×36979)×F_1 (Dorsett×37501)]	96.0
B_1 [F_1 (Fairfax×36979)×36979]	87.5
D. C. [F_1 (Fairfax×36979)×F_1 (Gem×361477)]	86.7
F_2 (Fairfax×36979)	79.8
Fragaria ovalis (36979), S_1	71.5
B_1 [Fairfax×F_1 (Fairfax×36979)]	59.4
F_1 (Fairfax×36979)	48.9
× *F. ananassa* (Fairfax), S_1	31.1

From these percentages and the calculations of table 2, it is apparent that the populations differ in the percentage of seeds that germinated during the 140-day period. Since the parents were not homozygous, and hence the F_1 populations might differ, it seemed probable that differences in percentage of germination might occur within populations. Such was found to be the case. However, the variation was not nearly so great within populations as between populations. Thus

it appears that variation due to segregation of genes within the parents was small as compared with that due to genetic differences between parents.

The F_1 (Fairfax $\times$ 36979) was used as the maternal parent in producing the seeds for the two double crosses, the backcross to 36979, and the F_2 populations; Fairfax was used as the maternal parent in producing the seed for the backcross to Fairfax, the F_1 progenies, and self-pollinated Fairfax; and of necessity, *F. ovalis* 36979 was used as the maternal parent in producing the seeds for self-pollinated 36979. When the F_1 hybrid was used as the female parent the percentage of the seeds that germinated was higher than when some other female parent was used. This would seem to indicate that the genotype of the maternal parent played a dominant role in determining the percentage of germination. If such actually were the case, there should be a difference between reciprocal crosses. The data on reciprocal crosses, given in table 3, show that when the F_1 hybrid was used as the female parent the percentage of germination in 140 days was 95.4, whereas when Fairfax was used as the female parent the percentage was 68.0. Of the seeds resulting from self-pollinating Fairfax, 62 percent germinated. It is evident that the genotype of the maternal parent played a dominant part in determining the percentage of germination.

With this fact established, the row totals for table 1 and the percentages for the different variates, previously listed, may be interpreted. The average percentage of germination for those populations having the F_1 hybrid as the female parent was 87.5; for those having Fairfax as the female parent, 54.2; for self-pollinated collection 36979, 71.5; and for self-pollinated Fairfax, 31.1. It is evident that heterosis exists as regards the effect of the maternal parent upon germination.

TABLE 3.—*Frequency distributions of germinating strawberry seeds approximately 2½ years old from reciprocal crosses and self-pollinated Fairfax*

Population	Seeds germinating after being in the germinator for the indicated number of days															
	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
F_1 (Fairfax $\times$ 36979) $\times$ Fairfax__	123	174	64	25	10	11	15	16	1	2	3	2	0	3	10	3
Fairfax $\times$ F_1 (Fairfax $\times$ 36979)__	3	45	56	17	10	9	14	21	12	6	6	6	2	10	38	17
Fairfax (S₁)_____	0	33	41	14	5	6	12	24	5	2	5	5	2	6	30	18
Total_____	126	252	161	56	25	26	41	61	18	10	14	13	4	19	78	38

Population	Seeds germinating after being in the germinator for the indicated number of days												Total	Germination	Mean time for germination
	85	90	95	100	105	110	115	120	125	130	135	140			
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Pct.*	*Days*
F_1 (Fairfax $\times$ 36979) $\times$ Fairfax____	3	1	4	0	1	2	2	2	0	0	0	0	477	95.4	18
Fairfax $\times$ F_1 (Fairfax $\times$ 36979)__	20	9	7	9	4	5	9	3	1	1	0	0	340	68.0	49
Fairfax (S₁)_____	21	13	16	4	5	15	11	4	10	2	1	0	310	62.0	60
Total_____	44	23	27	13	10	22	22	9	11	3	1	0	1,127	_____	_____

That the genotype of the maternal parent is not the sole factor controlling germination is shown by the fact that there were differences, not accountable for by chance variation, between germination percentages of the populations having the F_1 hybrid as the maternal parent. The range was from 79.8 to 96.0 percent. Moreover, the percentages of germination for the backcross to Fairfax and the F_1 were greater than that for self-pollinated Fairfax; yet Fairfax was the maternal parent of all of them. These data as a whole show that the genotype of those parts of the seed resulting from fertilization plays a part in determining the percentage of germination as well as does the genotype of the maternal parent.

The data for time of germination are given in table 1, and the heterogeneity x^2's for testing the significance of differences in table 2. There were marked differences between the means of hybrid populations and parents as to time of germination. This is also true, with the exception of the double cross [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Gem $\times$ 361477)], of the variates within hybrid populations. However, as noted in the case of percentages of germination, these differences are small as compared with the differences between means of hybrid populations and parents.

To facilitate the study of the data on time of germination the percentages of germinated seeds were calculated for the grouped subtotals (table 4). Without exception the populations having the F_1 hybrid as the maternal parent were the earliest to germinate and those having Fairfax as the maternal parent were the latest. These are the findings that would be expected if the maternal parent has a dominant influence on time of germination. To determine whether such was actually the case, reciprocal crosses involving the backcross to Fairfax were tested for time of germination (table 3). The data show that the backcross to Fairfax germinated considerably earlier when the F_1 hybrid was used as the female parent than when Fairfax was used as the female parent. Thus, from the data of tables 1 to 4, it may be concluded that the maternal parent had a predominant effect upon time of germination and that the F_1 hybrid exhibits decided heterosis in this respect.

TABLE 4.—*The percentage of the viable seeds that germinated during the indicated number of days after the seed had been put in the germinators (data from table 1)*

Population	Seeds germinating after being in germinator for indicated number of days								Mean for germination period
	40	45	50	55	60	65	70–85	90–140	
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Days*
D. C. [F_1(Fairfax$\times$36979)$\times$$F_1$(Dorsett$\times$37501)]	62.2	32.0	4.4	0.9	0.1	0.0	0.3	0.1	42
B_1 [F_1 (Fairfax$\times$36979)$\times$36979]	42.5	32.5	16.1	5.7	1.6	.3	.8	.5	45
F_2 (Fairfax$\times$36979)	29.8	40.4	19.0	4.8	2.3	1.8	1.1	.8	46
D. C. [F_1(Fairfax$\times$36979)$\times$$F_1$(Gem$\times$361477)]	14.2	46.1	27.5	7.1	3.0	.8	1.1	.2	47
Fragaria ovalis (36979), S_1	8.2	40.8	31.9	10.5	4.0	1.6	1.7	1.3	50
B_1 [Fairfax$\times$$F_1$ (Fairfax$\times$36979)]	3.5	11.9	20.4	15.5	9.0	11.9	14.7	13.1	64
F_1 (Fairfax$\times$36979), F_1 (Fairfax$\times$361477), and F_1 (Fairfax$\times$37501)	1.0	6.9	15.7	19.7	19.7	10.1	17.0	18.8	69
$\times$*F. ananassa* (Fairfax), S_1	2.3	5.3	9.1	15.2	15.9	12.9	24.2	15.1	69

That the genotype of those parts of the seed resulting from fertilization also had a decided effect upon time of germination is shown by the differences in time of germination between the populations having the F_1 hybrid as the maternal parent and again by the differences in time of germination between the populations having Fairfax as the maternal parent (table 4). The 2½-year-old seed started germination 35 days earlier than the 1-year-old seed. For the 2½-year-old seed the percentage of germination for the backcross to Fairfax was 8.6 percent higher than that of the 1-year-old seed, and for self-pollinated Fairfax, 30.9 percent higher.

It will be remembered that there were differences within populations as to time of germination, which would be due to genetic disparity within species. However, the variation due to differences in genotypes of the species was considerably greater than that due to genetic differences within species. The genetic differences within species probably were due to differences between varieties or collections of the species and to heterozygosity of these varieties or collections.

The foregoing studies on germination of strawberry seeds have a direct bearing upon a strawberry-breeding program. Reciprocal crosses do not necessarily behave alike and since the maternal parent has a preponderance of influence upon the percentage and time of germination, the breeder may well afford to determine which way his crosses should be made so as to give the greatest number of seedlings in the shortest time. It seems probable that in most crosses involving $\times$ *Fragaria ananassa* and *F. ovalis* the F_1 hybrid when used as the female parent will show heterosis as regards the maternal effect upon germination. So, other things being equal, time and labor may be saved by using the F_1 hybrids as the female parent in obtaining seeds for the production of the backcross generations.

Probably the most important result of these studies is the insight they furnish as to the possibilities for selection. As many as 96 percent of the seeds of some of the material germinated, and some were considerably earlier in germinating than others. This degree of genetic disparity in the material indicates that by selection in segregating hybrid populations the breeder may be able to build up stocks that will possess a high percentage of germination and will germinate quickly. This conclusion is in accord with the findings of Darrow et al. (*3*) and Henry (*8*). Their investigations show that both percentage and time of germination may vary according to species and variety.

FIELD STUDIES

As previously stated, the primary objective of the breeding program was to recombine the winter hardiness of the native Rocky Mountain strawberry with the fruit size of the cultivated varieties. Success in attaining this objective is affected by (1) the dominance and heterosis relations of the characters, (2) the frequency of occurrence of the desirable characters in the hybrid populations in which there is a possibility of these characters being recombined, (3) whether the genes differentiating the characters under consideration are independently inherited or are linked, (4) whether pleiotropy (multiple effects of a gene) is occurring as regards some or all of the genes differentiating certain characters, and (5) the effect of the environment on the rela-

tion between the characters.　Ten characters were selected for study. The data in respect to these are divided into three parts.　The first treats of dominance and heterosis, the second of the frequency of occurrence of the desirable character in the populations in which there is a possibility of these characters being recombined, and the third of the independence of the characters, of pleiotropy, and of the influence of the environment upon the relation between the characters.

DOMINANCE AND HETEROSIS

Probably one of the best ways of determining whether dominance and heterosis exist is to compare the means of the populations and parents.　Before proceeding further, it is desirable to have a clear conception of dominance and heterosis.　Even though, from the standpoint of physiological genetics, dominance and heterosis are probably fundamentally the same, it is convenient in these studies to use both terms.　Complete dominance is applied to those cases in which the phenotypic expression in the F_1 hybrid is that of one or the other parent.　The term "partial dominance" is used when the phenotypic expression in the F_1 hybrid lies between those of the parents but is anything other than exactly intermediate arithmetically.　Those cases are considered as exhibiting heterosis in which the phenotypic expression of a character in the F_1 hybrid is greater than the magnitude of such expression in both parents.　It should be pointed out that even though means of the F_1 compared with those for the parents provide the most information concerning dominance and heterosis, comparisons involving the means of other populations are valuable in furnishing supplementary evidence.

The means and their standard errors for the 10 characters studied are given in table 5.　The grades for winter injury listed in the second column show that in some cases the winter injury of the F_1 hybrid and of the double cross [F_1 (Fairfax×36979)×F_1 (Gem×361477)] is no greater than that of the respective *Fragaria ovalis* parents and in the remaining cases it is almost as low.　Hence, for all practical purposes the winter hardiness of the *F. ovalis* collections is almost if not completely dominant.　Differences in degree of winter hardiness exist within both *F. ovalis* and × *F. ananassa*, but these differences are minor as compared with the differences between the two.

All but two of the plants of Fairfax and all the plants of Dorsett winter-killed.　Hence, for these two varieties there were no strictly comparable data for the characters other than winter injury and number of runners.　Consequently, with the exception of the two characters mentioned, the means of Fairfax self-pollinated and Gem asexually propagated are used in the comparisons involving × *Fragaria ananassa*.

The data of table 5 show that the fruits of selfed 36979 averaged somewhat smaller than those of asexually propagated 36979, that the means of the F_2 populations were consistently lower than those of the F_1 populations, and that means of the double crosses were consistently greater than those of the F_2 populations.　However, in this latter comparison the differences were not marked.　Hence, under the environmental conditions encountered at Cheyenne large size of fruit is partially dominant.

TABLE 5.—*Means and standard errors of the populations*

(The larger the number the greater the degree of winter injury; the larger the size of the fruit; the greater the number of runners; the greater the sweetness of the fruit; the more desirable the flavor; and the firmer the flesh)

Population	Grade for winter injury	Grade for size of fruit	Plant height (Centimeters)	Grade for number of runners	Period from May 1 to first bloom (Days)	Period from first bloom to first fruit ripe (Days)	Period from May 1 to first fruit ripe (Days)	Grade for sweetness of fruit	Grade for flavor of fruit	Grade for firmness of fruit
Fragaria ovalis:										
37501 (asexual)	1.690±0.282	1.360±0.065	12.9±0.433	3.490±0.122	14.8±1.083	41.1±0.994	55.9±0.849	2.240±0.064	2.450±0.099	2.740±0.052
361477 (asexual)	.940± .133	1.044± .057	11.6± .400	3.380± .143	15.0±	43.4±1.473	63.4±1.481	2.390± .106	1.933± .091	2.967± .033
36979 (asexual)	1.000± .170	1.810± .057	11.3± .597	3.050± .114	14.0±	46.0± .943	60.2± .611	2.630± .097	2.700± .082	2.900± .037
36979 (S₁)	1.000± .124	.660± .037	8.8± .554	2.640± .103	14.5± .477	49.1± .605	63.6± .819	2.720± .074	2.700± .082	2.890± .048
Hybrid populations:										
B₁ [F₁ (Fairfax × 36979) × 36979]	1.070± .141	1.350± .040	16.3± .633	3.260± .070	14.3± .517	44.6± .702	58.9± .657	2.350± .090	2.460± .067	3.000± .068
F₁ [Fairfax × 36979]	1.320± .146	1.870± .073	17.7± .665	2.840± .085	16.0± .260	40.8± .389	57.1± .407	2.690± .105	2.670± .100	3.130± .033
F₁ [Fairfax × 361477]	1.030± .086	1.690± .077	19.1± .526	2.830± .078	17.4± .427	40.8± .573	58.2± .630	2.290± .063	2.430± .067	3.200± .037
F₁ [Fairfax × 37501]	1.300± .058	2.083± .065	18.7± .667	2.717± .095	18.0± .816	38.3± .615	56.3± .555	2.450± .123	2.550± .096	3.283± .099
F₂ [Fairfax × 36979]	2.310± .145	1.390± .066	13.4± .427	2.930± .040	23.2± .573	39.9± .605	63.1± .567	2.580± .076	2.630± .073	3.300± .037
F₂ [Fairfax × 36979]	1.920± .167	1.540± .043	13.2± .554	3.110± .115	21.3± .731	41.4± .452	62.7± .396	2.780± .047	2.900± .045	3.240± .048
F₂ [Fairfax × 36979]	1.870± .141	1.490± .072	12.7± .473	3.010± .085	22.5± .687	40.2± .291	62.7± .745	2.810± .050	2.850± .052	3.250± .054
D. C. F₁ (Fairfax × 36979) × F₁ (Dorsett × 37501)	1.540± .109	1.790± .059	19.4± .653	3.520± .093	17.0± .422	39.7± .651	56.7± .396	2.520± .070	2.610± .095	3.160± .037
D. C. F₁ (Fairfax × 36979) × F₁ (Dorsett × 37501)	1.660± .119	1.880± .061	20.3± .633	3.490± .050	16.7± .396	39.3± .517	56.0± .304	2.330± .078	2.410± .102	3.110± .041
D. C. F₁ (Fairfax × 36979) × F₁ (Dorsett × 37501)	1.610± .157	1.780± .074	19.0± .394	3.400± .086	17.0± .365	39.9± .277	56.9± .348	2.380± .081	2.520± .101	3.160± .056
D. C. F₁ (Fairfax × 36979) × F₁ (Dorsett × 37501)	1.580± .137	1.780± .071	19.8± .611	3.560± .087	16.5± .500	40.7± .539	57.2± .593	2.510± .083	2.550± .056	3.190± .043
D. C. F₁ (Fairfax × 36979) × F₁ (Gem × 361477)	1.050± .079	1.690± .028	15.5± .401	3.200± .097	17.4± .670	39.8± .663	57.2± .490	2.240± .054	2.350± .065	3.030± .033
D. C. F₁ (Fairfax × 36979) × F₁ (Gem × 361477)	1.080± .131	1.770± .073	14.2± .442	2.230± .063	16.8± .442	40.6± .521	57.4± .542	2.410± .059	2.660± .062	2.960± .052
D. C. F₁ (Fairfax × 36979) × F₁ (Gem × 361477)	1.340± .176	1.850± .089	14.8± .573	3.180± .074	18.3± .473	39.8± .416	58.1± .586	2.460± .037	2.550± .048	3.070± .042
B₁ [Fairfax × F₁ (Fairfax × 36979)]	2.750± .132	2.140± .075	13.9± .674	2.510± .084	25.3± .895	37.2± .611	62.5± .601	2.870± .082	2.950± .085	3.210± .050
B₁ [Fairfax × F₁ (Fairfax × 36979)]	2.790± .195	2.120± .098	13.8± .359	2.470± .092	25.7± .578	35.5± .795	61.6± .702	2.910± .066	2.080± .066	3.260± .022
B₁ [Fairfax × F₁ (Fairfax × 36979)]	2.840± .224	2.150± .075	14.8± .593	2.450± .096	24.5±1.046	35.9± .767	60.4± .806	2.880± .071	2.920± .090	3.300± .033
× *F. ananassa:*										
Fairfax (S₁)	4.650± .169	2.100± .243	9.6± .748	1.167± .084	31.8±2.332	33.0±1.265	64.8±1.900	2.880± .073	2.920± .097	3.280± .058
Fairfax (asexual)	5.100± .165			1.471± .097						
Gem (asexual)	4.210± .122	.900± .087	8.8± .512	1.330± .087	30.8±1.218	31.7± .651	62.5± .922	2.170± .054	2.290± .059	2.920± .039
Dorsett (asexual)	5.225± .111			1.025± .165						

From the data on plant height it can be seen that the backcross to *Fragaria ovalis*, the F_1 population, the F_2 population, both double crosses, and the backcross to Fairfax possessed greater height than did either Fairfax or 36979. Clearly plant height exhibits heterosis. The plants of the double cross [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Dorsett $\times$ 37501)] averaged as tall as those of the F_1 hybrids. This fact substantiates the field observations that the plants of this double cross were as vigorous as those of the F_1 hybrids. In fact, the extreme vegetative vigor of the F_1 populations and this double cross was so noticeable that statistical substantiation of this conclusion was not necessary. The purpose of making the double crosses was to allow for segregation of the genes differentiating the interspecific characters, but yet to retain as much as possible of any heterosis the F_1 hybrid might possess. It seems that the vegetative vigor of the F_1 hybrid can be retained by certain double crosses involving $\times$ *F. ananassa* and *F. ovalis*.

Turning to number of runners, it is clear, from column 5 of table 5, that the means of the F_1 hybrids approach more closely the means of the collection of *Fragaria ovalis* than they do those of the varieties of $\times$ *F. ananassa*. Hence, the tendency to produce a large number of runners is at least partially dominant to the tendency to produce a small number of runners.

For days from May 1 to first bloom the means of the F_1 hybrid approach rather closely those of the *Fragaria ovalis* collections, indicating a fairly high degree of partial dominance of early blooming. By the same criterion, the short period from first bloom to first fruit ripe is partially dominant, but the degree of dominance is slight. The addition of these two periods gives the days from May 1 to first fruit ripe, for which the F_1 hybrid exhibits a slight degree of heterosis. These conclusions regarding dominance and heterosis are substantiated by the fact that the means for the two double crosses are not materially different from those of their respective F_1 hybrids. Again, the desirable characteristics of the F_1 hybrids have been maintained in the double crosses. In days from May 1 to first bloom the collections of *F. ovalis* are earlier than the varieties of $\times$ *F. ananassa*, whereas in days from first bloom to first fruit ripe $\times$ *F. ananassa* has the shorter period. Consequently, the differences between the two species are slight as regards days from May 1 to first fruit ripe. This would indicate that the plant breeder may have considerable difficulty in combining these two phases of earliness.

For sweetness and flavor of fruit the data show the differences within species to be greater than the differences between species. For this reason the data are not conclusive in respect either to dominance or heterosis. However, one may conclude that in crosses involving *Fragaria ovalis* and $\times$ *F. ananassa* it should not be difficult to select plants possessing sweet fruits of high flavor.

Firmness of fruit presented no serious problem in these crosses. In general the fruits of the native Rocky Mountain strawberries used in the crosses were not as firm as those of the cultivated varieties. The means of the F_1 hybrid approached rather closely the mean of self-pollinated Fairfax, indicating at least partial dominance of firm fruit. However, as the differences between species were not very great this conclusion is of no great significance and probably not very well established.

<div align="center">Frequency of Occurrence of Characters</div>

The number of individuals in each class and the percentage of the individuals in the more desirable class, when the characters are divided into two classes, are listed in table 6. Chi square was used in testing the significance of differences noted (see Snedecor *16, pp. 19, 168–171*).

Plants shown in table 6 as having a grade for winter injury equal to or less than 0.5 or 1.0 are extremely winter hardy. In fact, a high degree of winter hardiness as compared with commercial varieties is possessed by any selection that averages 2.5 or less. Of the varieties of × *Fragaria ananassa*, only one plant (Gem asexually propagated) received a grade as low as 1.0. Of the segregating populations, the backcross to 36979 had the greatest proportion of its plants in the more desirable class for winter hardiness. However, the fruits of these plants were too small to be commercially acceptable. Next in order were the F_1 hybrids and the two double crosses. The F_1 hybrids and the double cross [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)] had somewhat more than 50 percent of their plants in the desirable class, whereas 40 percent of the plants of the other double cross fell in this class. As was to be expected of the hybrid populations, the backcross to Fairfax had the smallest percentage in the more desirable class. Probably the fact of greatest importance to a plant-breeding program was that plants possessing degrees of winter hardiness from 0.5 to 1.0 occurred frequently enough to allow selection for other characters.

The data on size of fruit (table 6) are of special interest because of the light they shed on the breeding behavior of this character. The greatest frequency of occurrence of plants having fruits of a size grade of 3.0 or greater was found in the F_1 population of Fairfax × 37501 and the backcross to Fairfax. Since Fairfax is the large-fruited parent, it would be expected that the highest percentage of large-fruited plants would be found in the backcross to Fairfax; but such was not the case. Why this particular F_1 hybrid should have had such a high proportion of large-fruited plants is not clear. A logical supposition is that *F. ovalis* may carry some genes for large size of fruit. However, in view of the work reported by Hildreth and Powers (*9*), this does not seem probable. Among the plants of 42,000 collections involving 1,100 locations, they found that none possessed fruits that would approach a size grade of 2.5. So large a number of collections from so many locations should form an adequate sample for testing what would be expected in the way of segregation within *F. ovalis*. Therefore, it seems probable that *F. ovalis* does not carry any genes for large size of fruit. Such being the case, it seems likely that the explanation is to be found in dominance relations. It will be remembered that large size of fruit was at least partially dominant. If in *F. ovalis* there were some genes that were completely or almost completely recessive to the genes of Fairfax for production of large fruit, then, by segregation within the *F. ovalis* parent, the large-fruited segregates obtained among the F_1 populations would be expected even though, comparatively speaking, no large-fruited *F. ovalis* plants have ever been found.

The question arises as to whether these large-fruited F_1 plants are true F_1 plants or false hybrids. The fact that they possess extreme winter hardiness and exhibit other characteristics of the *Fragaria*

TABLE 6.—*Classification of populations and percentage of individuals in the more desirable class when the characters are divided into 2 classes*

[Observational grades were used when the units of measurement are not stated]

Population	Winter injury			Size of fruit			Plant height in centimeters			Number of runners			Days from May 1 to first bloom			Days from first bloom to first fruit ripe			Days from May 1 to first fruit ripe			Sweetness of fruit			Flavor of fruit			Firmness of fruit		
	0.5–1.0 No.	1.5–5.5 No.	0.5–1.0 Pct.	0.5–2.5 No.	3.0–5.5 No.	3.0–5.5 Pct.	1–17 No.	18–31 No.	18–31 Pct.	0.5–3.0 No.	3.5–5.5 No.	3.5–5.5 Pct.	5–14 No.	17–62 No.	17–62 Pct.	18–33 No.	36–69 No.	18–33 Pct.	50–53 No.	56–89 No.	50–53 Pct.	0.5–3.0 No.	3.5–5.5 No.	3.5–5.5 Pct.	0.5–3.0 No.	3.5–5.5 No.	3.5–5.5 Pct.	0.5–3.5 No.	4.0–5.5 No.	4.0–5.5 Pct.
Fragaria ovalis:																														
37501 (asexual)	34	44	44	65	0	0	78	0	0	22	56	72	58	17	77	1	64	2	32	33	49	65	0	0	61	4	6	65	0	0
361477 (asexual)	71	22	76	27	0	0	93	0	0	44	49	53	68	25	73	0	31	0	2	29	6	75	0	0	74	1	4	27	0	0
36979 (asexual)	77	19	80	72	0	0	96	0	0	65	31	32	81	14	85	0	72	0	8	64	11	65	7	10	65	7	10	72	0	0
36979 (S1)	81	26	76	61	0	0	104	0	0	91	18	17	81	21	79	0	64	0	3	89	5	57	4	7	58	3	5	61	0	0
Hybrid populations:																														
B1 [F1 (Fairfax × 36979) × 36979]	84	31	73	106	5	5	74	39	35	51	65	56	23	23	80	0	106	0	23	83	22	104	2	2	94	12	11	101	5	5
F1 (Fairfax × 36979)	68	40	63	91	5	5	50	53	51	82	26	24	41	60	41	0	96	0	26	70	27	75	22	22	74	22	23	91	5	5
F1 (Fairfax × 361477)	80	37	68	110	3	3	33	84	72	99	18	15	42	74	36	11	111	16	15	98	13	110	3	3	101	12	11	104	12	18
F1 (Fairfax × 37501)	35	32	52	55	12	18	45	45	67	52	15	22	23	44	34	25	56	13	25	42	37	60	7	10	57	13	15	55	12	18
F2 (Fairfax × 36979)	110	234	32	258	3	1	301	36	11	226	118	34	35	264	12	33	229	13	11	252	4	229	32	12	201	60	23	230	30	12
D. C. [F1 (Fairfax × 36979) × F1 (Dorsett × 37501)]	184	275	40	409	27	6	122	337	73	151	308	67	221	226	49	34	402	8	159	277	36	402	34	8	355	81	19	400	36	8
D. C. [F1 (Fairfax × 36979) × F1 (Gem × 361477)]	226	122	65	319	18	5	272	75	22	185	163	47	134	210	39	24	313	7	98	239	29	317	20	6	289	48	14	327	10	3
B1 [Fairfax × F1 (Fairfax × 36979)]	39	205	13	189	41	18	240	53	18	288	16	5	16	240	6	65	166	28	30	201	13	175	55	24	155	75	33	210	20	9
× *F. ananassa:*																														
Fairfax (S1)	0	58	0	10	3	23	1	3	3	58	0	0	0	18	0	5	8	38	1	12	8	11	2	15	10	3	23	13	0	0
Fairfax (asexual)	0	15	0	2	2	23	0	2	0	15	0	0	0	2	0	5	8	100	0	2	0	2	2	2	2	2	3	0	0	0
Gem (asexual)	1	109	1	21	43	67	87	0	0	112	0	0	0	68	0	50	15	77	0	65	0	63	63	63	62	62	62	62	2	3
Dorsett (asexual)	0	21	0	0	0	0	1	0	0	21	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

ovalis parent, such as the ability to produce large numbers of runners, would rule out the probability of their being false hybrids. Further support of this conclusion is the fact that the selfed progeny of Fairfax had no plants that exhibited a high degree of winter hardiness.

The percentages for the different populations of the plants that would be expected to have both extreme winter hardiness and large size of fruits follow:

Population: *Percent*

B_1 [F_1 (Fairfax $\times$ 36979) $\times$ *Fragaria ovalis* 36979]_____ 0. 00
F_1 (Fairfax $\times$ *F. ovalis* 36979)_____ 3. 15
F_1 (Fairfax $\times$ *F. ovalis* 361477)_____ 2. 04
F_1 (Fairfax $\times$ *F. ovalis* 37501)_____ 9. 36
F_2 (Fairfax $\times$ 36979)_____ . 32
D. C. [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Dorsett $\times$ 37501)]_____ 2. 40
D. C. [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Gem $\times$ 361477)]_____ 3. 25
B_1 [Fairfax $\times$ F_1 (Fairfax $\times$ 36979)]_____ 2. 34

The most striking feature of these data is the theoretical possibility for selection among the F_1 plants of Fairfax $\times$ 37501. One other fact, which the data do not bring out, is that the fruits of the backcross to Fairfax falling in the desirable size group will average considerably larger than those for any of the other populations.

The greatest possibilities for selection of the desirable type for plant height, other considerations being equal, are offered by the F_1 hybrids and the double cross [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Dorsett $\times$ 37501)] (table 6). This was to be expected because of the greater heterosis shown by these populations. It is apparent that the possibilities are good for obtaining vigorous selections.

Among the populations having some large-fruited segregates, the chances of selecting plants producing a large number of runners are greatest for the double cross [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Dorsett $\times$ 37501)]. The other double cross ranks second in this respect. Again, the occurrence of the desirable type is sufficiently frequent to provide good opportunities for selection. It should be pointed out that under certain cultural practices the plants producing few runners are desirable. However, this is not true in the central Great Plains and Rocky Mountain regions.

The frequencies of occurrence of the classes for the different phases of earliness will be considered together. Among the segregating populations having some plants with large fruits, the F_1 plants and the double crosses offered the greatest possibilities for selecting those having a short period from May 1 to first bloom. This was not true for days from first bloom to first fruit ripe, as the backcross to Fairfax had the largest percentage of plants in the desirable class. For days from May 1 to first fruit ripe the double crosses and the two F_1 populations involving 36979 and 37501 had the largest percentage of plants in the desirable class.

In considering sweetness, flavor, and firmness of fruit it should be pointed out that the two species did not differ greatly except possibly in respect to firmness (table 5). The parents from both species, with

the possible exception of Gem and collection 361477, possessed high-quality fruit. The more desirable classes for which the percentages are given represent the plants having extremely high-quality fruits; hence many plants in the other class had fruits of very good quality. In fact, quality in these hybrids is a minor problem as the majority of segregates had fruits of good quality. Whenever Gem, 361477, or both entered into a cross the proportion of plants having extremely high quality was reduced (table 6).

<div align="center">FREQUENCY OF RECOMBINATION OF DESIRED CHARACTERS</div>

If the relation of two characters is such that they are independent in the segregating populations, then the frequency of the occurrence of individuals recombining the two characters can be predicted. For example, the proportion of the plants of the double cross [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Dorsett $\times$ 37501)] having a grade of 1.0 or less for winter injury was 0.400871 and the proportion of the plants of the same cross having a plant height falling within 18 to 31 cm. was 0.734205. The expected proportion of the total number of plants that recombined both characters is, then, 0.400871 $\times$ 0.734205, or 0.294321; and the expected proportion that possessed the alternative characters is 0.705679. Chi square was employed to test whether the differences between the expected numbers and those obtained could be accounted for by chance.

In obtaining these χ^2 values, when consistent with the biological considerations of table 6, the data were grouped so that the expected number for either of the two classes was not less than five. When this was not advisable the difficulties arising from small numbers (*6, p. 96*) were taken into consideration. The χ^2 values are listed for each category of table 7. Both are necessary for a complete analysis of the data. One of the advantages of the second χ^2 is that the expected numbers are large.

If the differences between the obtained and expected numbers cannot be attributed to chance variation, then linkage, pleiotropy, environment, or a combination of these may be responsible for the discrepancies noted. By calculating the χ^2 values for all the populations of table 6, information may be obtained as to which of these factors are operating to produce the discrepancies noted. All these χ^2 values were calculated, but only those of the five populations segregating for the genes differentiating the two species are given in table 7. The data of this table will be discussed on the basis of the row designations. For example, when discussing the χ^2 values for the row designated winter injury the χ^2 values for all the other characters and winter injury are discussed; whereas for size of fruit the χ^2 value for this character and winter injury are not included, as it would have been discussed previously under the row designated winter injury.

TABLE 7.—χ^2 *values for testing independence of the different characters calculated from the populations segregating for the genes differentiating the species*

[The χ^2 listed first under each column and each row is the sum of the χ^2's for the individual generations and has 5 degrees of freedom; whereas the χ^2 listed second under each column and each row is calculated from the totals of the expected and the obtained for all the populations and has but 1 degree of freedom]

Character	Size of fruit	Plant height	Number of runners	Days from May 1 to first bloom	Days from first bloom to first fruit ripe	Days from May 1 to first fruit ripe	Sweetness of fruit	Flavor of fruit	Firmness of fruit
Winter injury	6.787	32.528*	6.121	6.902	1.858	7.372	4.438	3.914	5.585
	.393	11.936*	2.714	3.070	.201	3.413	2.516	1.915	3.637
Size of fruit		5.005	2.216	19.139*	30.908*	244.281*	13.416*	13.090*	1.123
		1.558	.181	13.618*	18.656*	194.811*	8.105*	8.722*	.426
Plant height			15.730*	11.691*	1.939	5.727	4.974	2.268	3.281
			6.559*	3.012	.001	2.270	.246	.018	1.718
Number of runners				16.805*	1.857	9.474	2.013	.895	2.588
				8.557*	1.105	3.587	.534	.288	.318
Days from May 1 to first bloom					68.877*	93.669*	2.555	2.305	1.510
					66.294*	42.556*	.344	.990	.402
Days from first bloom to first fruit ripe						36.510*	4.582	2.915	.553
						28.535*	2.235	1.615	.020
Days from May 1 to first fruit ripe							3.603	5.101	12.231*
							.909	1.365	5.792*
Sweetness of fruit								295.546*	.484
								224.200*	.138
Flavor of fruit									1.142
									.610

*$P<0.05$, indicating that the deviations noted cannot be attributed to chance.

From table 7 it can be seen that with the exception of plant height none of the χ^2 values for winter injury and the other characters are of sufficient magnitude to warrant the conclusion that the genes differentiating them are not independently inherited. It remains to be seen whether linkage, pleiotropy, environment, or a combination of these factors is responsible for the association noted between winter injury and plant height. Information pertaining to this phase of the problem may be obtained from table 8. The statistically significant χ^2 values are for those populations in which the proportion of non-winter-hardy plants would be expected to be greatest; namely, Gem, selfed Fairfax, the F_2 populations, and the backcross to Fairfax. The discrepancies noted for asexually propagated Gem cannot be attributed to linkage or pleiotropy. However, the facts observed are logically explained as due to environmental differences (winter injury to some plants was so severe as to reduce their height materially). If this deduction is correct, the obtained number for those populations having statistically significant χ^2's should in every instance exceed the expected. It will be noted that such is the case. Such a relation between the two characters does not affect the ability of a breeder to obtain the desired type by selection, because the environment affects the expected and not the obtained from which the breeder makes the selections. In other words, the environment has lowered the actual proportion of tall plants at the expense of the genetically potentially tall plants of the non-winter-hardy group. Since the expected number of plants combining hardiness and tallness of plant is calculated by multiplying the total proportion of tall plants expressed as a decimal fraction by the total proportion of winter-hardy plants expressed as a decimal fraction and this resultant fraction by the total number of individuals in the population, it is apparent that the expected has been lowered by this environmental action, whereas the obtained has

not. Therefore, as previously stated, the plant breeder has the same number and kind of individuals to select from as would have been the case if the environment had not had the effect noted.

TABLE 8.—χ^2 *values for testing whether the differences between the numbers obtained and those expected, if winter injury and plant height are independent, can be attributed to chance*

Population	Total plants	Plants combining winter hardiness and tallness		χ^2
		Obtained	Expected	
	Number	*Number*	*Number*	
Fragaria ovalis (37501), asexual	78	11	11.8	0.059
F. ovalis (361477), asexual	93	11	12.2	.139
F. ovalis (36979), asexual	96	14	14.4	.016
× *F. ananassa* (Gem), asexual	85	10	4.6	6.658*
Sum of χ^2's				6.872
χ^2 of totals	352	46	43.0	.233
F. ovalis (36979), S$_1$	104	8	6.1	.663
F$_1$ (Fairfax×36979)	103	38	33.4	.950
F$_1$ (Fairfax×37501)	67	22	23.5	.149
F$_1$ (Fairfax×361477)	117	59	57.4	.084
× *F. ananassa* (Fairfax), S$_1$	35	8	2.5	13.197*
Sum of χ^2's				15.043*
χ^2 of totals	426	135	122.9	1.688
B$_1$ [F$_1$ (Fairfax×36979)×36979]	113	29	28.5	.012
F$_2$ (Fairfax×36979)	337	21	11.5	8.097*
D. C. [F$_1$ (Fairfax×36979)×F$_1$ (Dorsett×37501)]	459	152	135.1	2.998
D. C. [F$_1$ (Fairfax×36979)×F$_1$ (Gem×361477)]	347	59	48.7	2.531
B$_1$ [Fairfax×F$_1$ (Fairfax×36979)]	293	18	6.8	18.889*
Sum of χ^2's				32.527*
χ^2 of totals	1,549	279	230.6	11.936

*$P<0.05$, indicating that the deviations noted cannot be attributed to chance.

Turning to size of fruit, it can be seen that all the χ^2 values other than those for plant height, runners, and firmness of fruit are statistically significant. Again, in every case, the number of individuals obtained for the class combining the desirable characters is greater than the number expected. The explanation for those characters that determine earliness of maturity seems to be that the environment has a similar effect upon them and size of fruit. It is evident that linkage of the genes that differentiate size of fruit with those that differentiate the earliness characters cannot be the explanation. On the other hand, the relation between size of fruit and sweetness and size of fruit and flavor seems to be due to linkage of the genes differentiating these characters, to pleiotropy, or to both.

The χ^2 values for plant height and number of runners are statistically significant. The sum of the χ^2's for plant height and days from May 1 to first bloom is also statistically significant, and the χ^2 for totals approaches statistical significance, if a P value of 0.05 or less is accepted as a criterion of statistical significance. Again, for the desirable combination of characters the obtained is greater than the expected. Since all of these characters are measures of plant vigor, it is not surprising that this is so. It would seem that pleiotropy surely is occurring, and linkage also would be expected to play some part. That none of the χ^2 values for the asexually propagated generations approached statistical significance supports these statements and

furnishes convincing evidence that the causes of these high χ^2 values are genetic. In any event, whether linkage or pleiotropy is responsible for the high χ^2 values, the task of the plant breeder is easier because of the greater proportion of plants combining the desirable characters from which selections can be made.

For number of runners, the only statistically significant χ^2 values are those listed opposite days from May 1 to first bloom. As before, the obtained class recombining the desirable characters is larger than the expected. The explanation is the same as that given in the preceding paragraph for plant height, runners, and days from May 1 to first bloom. In fact, this relation between runners and days from May 1 to first bloom was to be expected. It aids rather than hinders the breeding program.

Days from May 1 to first bloom, from first bloom to first fruit ripe, and from May 1 to first fruit ripe will be considered together. None of the χ^2 values for these characters and sweetness, flavor, or firmness of fruit is statistically significant, and hence will not be considered further. The χ^2 value for days from May 1 to first bloom and days from May 1 to first fruit ripe is statistically significant, as is also the χ^2 value for days from first bloom to first fruit ripe and days from May 1 to first fruit ripe. In all of these cases the obtained numbers of the desired class are greater than the expected numbers. None of the corresponding χ^2 values for the asexually propagated material were statistically significant. These facts show that the causes are genetic. These causes would be expected to be genetic since days from May 1 to first bloom plus days from first bloom to first fruit ripe gives days from May 1 to first fruit ripe. The χ^2 values for days from May 1 to first bloom and days from first bloom to first fruit ripe are statistically significant. From table 9, which gives all the χ^2 values for these two characters, it can be seen that none of the χ^2 values for the asexually propagated populations was statistically significant. For the first and only time the expected numbers were larger than those obtained for the desirable class. Since the genes differentiating the desirable characters entered the cross from different parents, it would seem that linkage, pleiotropy, or both are responsible for the high χ^2 values obtained. In any event the task of combining these two characters into a single individual to obtain a very early type with other desirable characters is much more difficult than if the genes differentiating the two characters had been independently inherited.

TABLE 9.—χ^2 *values for testing whether the differences between the numbers obtained and those expected, if days from May 1 to first bloom and days from first bloom to first fruit ripe are independent, can be attributed to chance*

Population	Total plants	Plants combining short period from May 1 to first bloom and short period from first bloom to first fruit ripe		χ^2
		Obtained	Expected	
	Number	*Number*	*Number*	
Fragaria ovalis (37501), asexual	65	3	4.6	0.624
F. ovalis (361477), asexual	31	6	5.8	.005
F. ovalis (36979), asexual	72	1	2.6	.984
× *F. ananassa* (Gem), asexual	65	35	36.5	.135
Sum of χ^2's				1.748
χ^2 of totals	233	45	49.5	.523
F. ovalis (36979), S_1	64	6	6.4	.022
F_1 (Fairfax × 36979)	96	0	5.3	5.584*
F_1 (Fairfax × 37501)	67	3	10.3	6.112*
F_1 (Fairfax × 361477)	113	0	8.3	8.990*
× *F. ananassa* (Fairfax), S_1	13	7	5.6	.656
Sum of χ^2's				21.364*
χ^2 of totals	353	16	35.9	12.198*
B_1 [F_1 (Fairfax × 36979) × 36979]	106	0	.8	.803
F_2 (Fairfax × 36979)	262	2	9.0	5.652*
D. C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	436	19	61.3	33.972*
D. C. [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)]	337	9	33.5	19.896*
B_1 [Fairfax × F_1 (Fairfax × 36979)]	231	0	8.2	8.555*
Sum of χ^2's				68.878*
χ^2 of totals	1,372	30	112.8	66.294*

* $P < 0.05$, indicating that the deviations noted cannot be attributed to chance.

Sweetness, flavor, and firmness of fruit will be discussed together. None of the χ^2 values for firmness of fruit with either of the other two characters was statistically significant. Hence, the hypothesis of independence of this character of the other two was not disproved. The χ^2 values for sweetness and flavor of fruit were statistically highly significant. This was also true for the asexually propagated parents, showing that environment exerts some effect upon the χ^2 values. Again, the obtained numbers for the class combining the desirable characters were larger than the expected. How much, if any, of this relation can be attributed to linkage and pleiotropy cannot be ascertained from these data.

To summarize, it can be said that in only 1 of the possible 45 cases did linkage or pleiotropy interfere with the breeding program and in only 14 of the possible cases were the characters other than independent.

DISCUSSION AND CONCLUSIONS

PROMISING SELECTIONS

From the crosses between $\times$ *F. ananassa* and *F. ovalis*, 74 selections having large fruits and a high degree of winter hardiness have been chosen for increase and further testing. Already 3 of them have shown sufficient promise to warrant their release. Descriptions of these, the parents, and Gem are given in table 10.

During the past two winters all three selections have proved to be winter hardy. Their fruits are large and of very good quality. The fruits of the two F_1 hybrids are of good size but somewhat smaller than those of the cultivated varieties. This somewhat smaller size is more than compensated for, however, by their hardiness and earliness. The backcross to Fairfax has fruits as large as those of Dorsett and Fairfax but somewhat smaller than those of Gem. In addition, it is more prolific than either Dorsett or Fairfax. All three selections produced more runners than either Gem, Dorsett, or Fairfax, and the fruits of the two F_1 selections are very aromatic. The differences in leaf spotting between the selections and cultivated varieties were slight, the cultivated varieties being somewhat freer from leaf spots. Leaf spotting is not sufficiently serious in either to be of economic importance under the conditions existing at Cheyenne. The quality of these selections as regards sweetness and flavor is equal if not superior to that of Fairfax. The fruits of the F_1 selections are not quite so firm as those of Fairfax, and probably they would not be good shippers. However, the fruits of the backcross to Fairfax are firm and this selection should ship well.

TABLE 10.—*Description of the characters of 3 selections from crosses involving cultivated varieties and the native Rocky Mountain strawberry, 3 standard varieties, and 2 collections of Fragaria ovalis*

Selection, variety, or collection	Grade for winter injury	Grade for size of fruit	Height of plant	Grade for number of runners	Period from June 1 to first fruit ripe	Grade for sweetness of fruit	Grade for flavor of fruit	Grade for firmness of fruit	Grade for prolificacy of fruit	Grade for leaf spotting
			Centimeters		*Days*					
Fragaria ovalis:										
361261	0.5	1.0	14	3.0	22	2.0	2.5	2.5	2.0	2.0
36979	.5	1.0	14	3.5	23	2.7	2.7	2.9	2.0	2.0
Selections from hybrid populations:[1]										
F_1 (Dorsett $\times$ 361261)	1.0	2.8	17	3.5	10	3.5	3.5	3.5	4.1	1.0
F_1 (36979 $\times$ Fairfax)	1.0	3.0	19	3.2	21	3.8	3.7	3.5	2.5	1.0
Backcross [Fairfax $\times$ F_1 (Fairfax $\times$ 36979)]	1.2	3.5	22	3.0	34	3.5	3.5	4.5	3.5	1.0
$\times$ *F. ananassa:*										
Fairfax	4.8	3.5	12	1.5	36	3.5	3.5	4.0	2.5	.5
Dorsett	4.8	3.5	12	1.5	36	3.2	3.0	4.0	2.5	.5
Gem	4.0	4.0	10	2.0	34	2.1	2.5	4.0	3.5	.5

[1] The varietal names given to these 3 selections in order of their mention are Early Cheyenne 1, Cheyenne 2, and Cheyenne 3.

As the testing of the 74 selections progresses, it seems highly probable that some may prove superior to the 3 described. Furthermore, additional study of the segregating populations may bring to light plants superior to the selections already made. In selecting further, the breeder should give particular attention to the everbearing habit.

EVALUATION OF BREEDING METHODS

The question may be asked whether the broad method of breeding adopted, namely, that of hybridizing before inbreeding, was justified. The genetic disparity between species as regards the characters differentiating winter injury and size of fruit was so much greater than that within species as to render the latter negligible in accomplishing the principal objective of the breeding program. This fact alone justifies the adoption of the method employed. The same conclusion is warranted for plant vigor as represented by plant height, ability to produce runners, days from May 1 to first bloom, days from first bloom to first fruit ripe, and days from May 1 to first fruit ripe. Such, however, was not the case with the characters that influence quality, namely, sweetness, flavor, and firmness of fruit. However, in the central Great Plains and Rocky Mountain regions, quality as compared with winter hardiness is not a major problem. Therefore, it must be concluded that employing the broad method involving hybridization before inbreeding was fully justified.

Since hybridization before inbreeding was found to be the most promising method of obtaining immediate practical results, the next step was to determine which populations and lines of breeding offered the most promise. The F_1 hybrid of Fairfax $\times$ 37501 had the greatest number of plants combining extreme winter hardiness and fruits sufficiently large to be acceptable for home garden production. This was not true of the F_1 hybrids involving Fairfax and collections 36979 and 361477, a fact which shows that the frequency of occurrence of large fruit is also dependent upon the genotype of the collection of *F. ovalis*. Such being the case, further breeding programs should include the production and testing of a large number of F_1 hybrid plants involving the crossing of a number of collections with a number of cultivated varieties. The fundamental principle justifying such a procedure is that genes are present in some collections of *F. ovalis* which in the F_1 hybrid of crosses with $\times$ *F. ananassa* allow the almost complete if not the complete expression of the genes for large size of fruit. The only means of locating these collections of the native Rocky Mountain strawberry which carry such genes is by breeding tests such as those outlined above.

Backcrossing the F_1 hybrid to the cultivated parent has given very good results also. In fact, these results are even better than the data reported in table 6 would indicate. The reason for this is that the size of fruits falling in the desirable class will average larger for the backcross to Fairfax than will the F_1 fruits falling in this same class. Because of this fact, it seems that the backcross method probably offers the greatest possibilities for the most rapid accomplishment of the objectives of the breeding program. Further work therefore should involve backcrossing to the cultivated parent and also outcrossing to a number of the other cultivated varieties. However, in following the backcross and outcross methods of breeding, a more rigid selection of the F_1 plants should be made so as to obtain larger fruited F_1 plants than were available when the breeding program was started at Cheyenne. Also, some of the more promising selections from the backcross to Fairfax should be used in further backcrossing, in crossing among themselves, and in outcrossing to other cultivated varieties possessing special merit.

The double crosses are particularly promising because of the comparatively large proportion of the population possessing winter hardiness, because of the marked vegetative vigor of some of the double crosses, and because of the outstanding productiveness of some of the plants. Again large-fruited F_1 hybrids should be selected for making the crosses. If this is done there seems to be no reason why the plant breeder should not obtain extremely winter-hardy, large-fruited, and very prolific strawberries that would not only excel the present commercial varieties but also the selections already made from the breeding material involving the hybridization of $\times$*Fragaria ananassa* and *F. ovalis*. Also, outstanding double-crossed plants should be used in crossing among themselves and in outcrossing with existing varieties possessing special merit.

The F_2 population did not offer as much opportunity for selection as the other hybrid populations. This may have been partly due to the reduction of vigor in the F_2 population as compared with the F_1 hybrids and the double crosses. Table 5 shows that the size of fruits of the *Fragaria ovalis* collection 36979 inbred by self-pollination for one generation did not average as large as the fruits of the parent asexually propagated. The same was true of the fruits of the F_2 plants as compared with the fruits of the corresponding F_1 plants. Thus it would seem that when maximum segregation is desired double crosses offer far more promise than F_2 populations. This certainly was true in the studies reported herein. However, crossing among selected F_2 plants, outcrossing to meritorious existing varieties, and crossing with selections from other generations should produce selections superior to those now available.

BREEDING PRINCIPLES

The extremely infrequent occurrence of individuals recombining all the desirable characters is emphasized by the data for the double cross [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Dorsett $\times$ 37501)]. Assuming independent inheritance, to recombine all the desirable characters given in table 6 into a single individual would, on the average, require a population of 5 million plants. However, if the breeder is content with extreme winter hardiness, large fruit, exceedingly vigorous plants, ability to produce a large number of runners, and a date of maturity and quality of fruit similar to that of Dorsett and Fairfax, he may find such a plant on the average in every 100 plants of this double-cross population. This last statement explains why it was possible to obtain 74 promising selections from the breeding material involving crosses between the cultivated varieties and the native Rocky Mountain strawberry. The reason why so many plants, comparatively speaking, recombine these 4 desirable characters is that winter hardiness and ability to produce runners are almost completely dominant; larger size of fruit shows a small degree of partial dominance; and plant height shows a high degree of heterosis.

SUMMARY

This paper reports the results of breeding studies involving crosses between the cultivated varieties ($\times$ *Fragaria ananassa*) and the native Rocky Mountain strawberry (*F. ovalis*). The material included the parents asexually propagated, the progeny obtained by

selfing the parents, the progeny obtained by backcrossing to both parents, the F_1 hybrids, the F_2 population, and the double crosses.

The percentage of strawberry seeds germinating varied from 31.1 percent for selfed Fairfax to 96 percent for the double cross [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Dorsett $\times$ 37501)]. The mean number of days required for the percentages of germination noted above was 69 for selfed Fairfax and 42 for the double cross.

Reciprocal crosses did not behave alike in respect to percentage or time of germination. The maternal parent was found to have a preponderance of influence. The percentage of germination was greatest and the time required for germination least when the F_1 generation was used as the female parent. In fact, the F_1 hybrid showed heterosis as regards this matroclinous effect.

The genetic disparity in the material indicates that by selection in segregating hybrid populations the breeder can build up breeding stocks that will show a high percentage of germination and that will germinate quickly.

The following characters showed either partial or complete dominance: A high degree of winter hardiness over winter injury; large over small size of fruit; a large over a small number of runners; a short over a longer period from May 1 to first bloom; and a short over a longer period from first bloom to first fruit ripe. Height of plant and the short period from May 1 to first fruit ripe showed heterosis. In both cases, this heterosis was retained by the double cross [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Dorsett $\times$ 37501)]. The data on quality of fruit (sweetness, flavor, and firmness) were not conclusive as regards dominance and heterosis.

The data on the frequency of the occurrence of plants possessing the more desirable of the contrasted characters showed that the opportunity for selecting the desired types was greatest among the plants of the backcross to Fairfax, certain F_1 hybrids, and the double crosses.

The studies on the frequency of the occurrence of plants recombining desirable characters show that only in the case of days from May 1 to first bloom and days from first bloom to first fruit ripe did genetic linkage reduce the number of plants combining the desirable characters; that is, the number of such plants obtained was less than the number expected if the genes differentiating the contrasted characters are independently inherited. In the other 13 cases of nonindependence of the characters, the relations found either helped in obtaining the desired combinations of characters or had no effect. In 31 of the possible 45 cases the characters were found to be independent.

On an average, 1 individual per 100 was found to recombine the following desirable characteristics: Extreme winter hardiness, large fruits, exceedingly vigorous plants, ability to produce a large number of runners, and a date of maturity and quality of fruit similar to those of Dorsett and Fairfax.

Seventy-four promising selections were obtained from the breeding material involving crosses between the cultivated varieties and the native Rocky Mountain strawberry. The reason why it was possible to obtain so many plants, comparatively speaking, that recombine the desirable characters is that winter hardiness and the ability to

produce runners are almost completely dominant, size of fruit shows a small degree of partial dominance, and plant height and short period from May 1 to first fruit ripe exhibit heterosis.

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ALFALFA SEED PRODUCTION AS AFFECTED BY ORGANIC RESERVES, AIR TEMPERATURE, HUMIDITY, AND SOIL MOISTURE [1]

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INTRODUCTION

In many parts of the world where alfalfa (*Medicago sativa* L.) is grown seed production is poor, whereas in other areas the seed comprises the major value of the crop. Climatic conditions are usually assumed to account for the differences. The object of the investigations reported here was to determine the extent to which organic reserves in the roots, soil moisture, and climatic factors, such as air temperature and relative humidity, affect seed setting. It is thought that external factors may influence seed production in alfalfa directly by affecting the development of top growth and the viability and growth of pollen and indirectly by affecting the internal factors of plant-food manufacture and storage. Experiments were started in 1934 at the Kansas Agricultural Experiment Station, Manhattan, Kans., to determine the importance of these factors and to ascertain whether certain treatments correlate with high seed production.

REVIEW OF LITERATURE

Many factors affecting seed development in plants have been studied. Hollowell (9)[2] stated that atmospheric humidity does not affect the setting of red clover seed in the greenhouse or field and that neither high nor low soil moisture prevents seed setting. However, he stated that plants grown with the soil moisture slightly below the optimum for growth matured flowers more satisfactorily for seed production.

Alter (1) stated that a certain amount of stress on the alfalfa plant was necessary to force the setting of seed. Observations have indicated that the production of alfalfa seed is best in areas where the air humidity is usually low and the soil moisture below optimum. Blinn (3) reported that good yields of beet seed were obtained by holding the irrigation water near the minimum requirement of the plant; he concluded that dry climatic conditions with high temperatures seem to be among the most essential requirements for seed production.

Carlson (5) stated that a desertlike climate, especially one with light but frequent summer rains, is considered a factor influencing

alfalfa seed production in the valley lands of Utah. He also expressed the opinion that the frequent rains conditioned the atmosphere rather than the soil. Fuelleman [3] studied the effects of water utilization, organic reserves, and nitrogen supply on seed production in alfalfa and concluded that extreme retardation of vegetative vigor favored seed formation more than extreme acceleration of vegetative vigor. Nightingale and Farnham (*10*), working with sweet peas in the greenhouse, found that if water was used sparingly the salt concentration of the soil solution was increased, thereby limiting the absorption of water, inducing early maturity of tissue, and decreasing the rate of protein synthesis from nitrates. As a result carbohydrates accumulated, as they were used less extensively in protein synthesis. These authors stated further (*10, p. 512*): ". . . to obtain ideal vegetative and reproductive growth, the protein nutrition of plants must be considered in relation to opportunity for carbohydrate synthesis."

Garner et al. (*6*) noted the changes taking place in the carbohydrates in the upper parts of the stems of cosmos plants during short intervals. With Biloxi soybeans in the bud stage they found that reducing sugars increased during late summer and early fall, decreased when blossoms appeared, and increased again when seeds were developing. Soluble nitrogen also increased in the seed stage.

The period from the primordial stage of the alfalfa flower through the development of the seed may include 25 to 45 days. During that time the external factors may have changed several times, thus affecting the internal factors. Bolton and Fryer (*4*) noted such changes and stated that internal plant factors may not promote seed setting in alfalfa as definitely in the later stages as in the earlier stages of the flowering period.

From this partial review of the literature it is evident that external factors affect the seed production of certain plants and may do so in part by altering the internal factors. It is essential that more be known about the extent to which each factor affects production and how the external factors are related to the internal factors.

METHODS OF EXPERIMENTATION

Two controlled experiments were set up for these investigations, one for studying the influence of air temperature and relative humidity on seed production and the other for studying the influence of soil moisture and organic reserves. The chamber used for the control of air temperature and relative humidity, in which sulfuric acid was used to control humidity, was constructed and described by Grandfield and Zink (*8*). This equipment permitted the studies to be carried on in natural light under greenhouse conditions. To eliminate the inherent variability of different alfalfa plants, cuttings tracing back to a single plant were used each year in all experiments. The alfalfa plants were established in 6-inch clay pots and allowed to develop to the full-bloom stage. At the time the plants were to be placed in the chamber, the older flowers and buds were removed from each raceme, the remaining flowers were tripped and counted, and each raceme was tagged. In the course of the experiment different plants were exposed to various temperatures ranging from

[3] FUELLEMAN, R. F. WATER UTILIZATION AND OTHER FACTORS INFLUENCING THE FRUITING OF ALFALFA. Univ. of Wis. 2 pp. 1934. [Processed.

60° to 120° F. and to relative humidities ranging from 10 to 90 percent. It was learned from earlier trials that exposures of from 3 to 8 hours in the cabinet gave satisfactory results. After exposure the plants were returned to the greenhouse, where the seeds were allowed to develop. A record was made of the number of flowers tripped and of the pods and seeds formed.

The experiment on soil moisture and organic reserves was conducted by means of special equipment developed by the author (7) for this study. The soil was prepared by mixing five parts of Wabash silt loam and one part of sand with a small amount of compost. This mixture allowed water to penetrate readily and had a high water-holding capacity. The moisture equivalent and the wilting coefficient were determined for each experiment in the usual manner. The field water-holding capacity of the soil was determined by placing 12-inch columns of soil 1 inch in diameter in a perpendicular position on sand, saturating the soil with water, and allowing the columns to drain for 24 hours before determining the percentage of moisture. This percentage was assumed to represent the field water-holding capacity of the soil.

The experiment was conducted under two levels of soil moisture, referred to in this paper as "high" and "low." The high level was near the field water-holding capacity (22 percent); the low level was 12 percent, being 4 percent above the wilting coefficient. The watering was done as described by the author (7), and the pots were brought to the proper weight each day. The scales used permitted the pots to be weighed to an accuracy of ± 10 gm. One hundred pots were used in this experiment, with four combinations of soil moisture and food reserves as follows: Low moisture, low reserves; high moisture, low reserves; low moisture, high reserves; and high moisture, high reserves.

The high and low reserves were obtained by keeping the top growth cut back on plants in half the pots to reduce the reserves, and allowing the plants in the other pots to grow normally to the full-bloom stage. While the plants were becoming established and the reserves were being depleted in half of them, the soil moisture was held at 15 percent. After the plants had become established and the differences in reserves had been obtained, all the top growth was removed. Half of both the low-reserve and the high-reserve plants were reduced to the 12-percent soil-moisture level, and half were increased to the 22 percent level to obtain the four conditions just mentioned. As the plants came into bloom, each raceme was tagged and a record was made of the number of racemes and the number of flowers tripped, pods formed, and seeds developed.

Laboratory determinations for total carbohydrates were made by the iodometric method of Shaffer and Hartmann (11), and the amounts were calculated as dextrose from the Munson-Walker tables (2). Total nitrogen content was determined by the Kjeldahl method.

A soil-moisture-control and time-of-irrigation experiment was conducted by varying the amount of water supplied to the plants during seed-crop development and the seeding period. Four moisture treatments were used: (1) Low moisture; (2) low moisture from the time growth started on seed crop until prebud stage, with high moisture during the flowering and ripening period; (3) high moisture through-

out; and (4) high moisture until top growth was well started, low moisture until prebud stage, and then high moisture until seed was matured. High moisture was at 22 percent and low moisture at 12 percent, as in the previous experiment.

EXPERIMENTAL RESULTS

Preliminary work was started on the experiments in 1934, but, owing to the necessity of developing special equipment, definite results were not obtained until 1936. The investigations of the various factors affecting the production of alfalfa seed will be discussed under two headings, namely, Influence of Air Temperature and Relative Humidity, and Influence of Soil Moisture and Organic Reserves.

INFLUENCE OF AIR TEMPERATURE AND RELATIVE HUMIDITY

Air temperature, relative humidity, and soil moisture are closely associated in their interaction on crop plants, and conclusions cannot be drawn without taking all these factors into consideration. The combined effect of temperature and humidity under comparable soil-moisture conditions on the number of flowers setting pods is shown in table 1.

Under the conditions of this experiment very few alfalfa flowers set pods at temperatures above 100° F., regardless of the relative humidity. This is contrary to common observation, because alfalfa plants do set pods in the greenhouse where air temperatures are as high as 120°. The plants used were not hardened to high temperatures but were taken from the greenhouse bench where the air temperature ranged from 70° to 90°. Therefore, the maximum temperature for the proper functioning of the flower parts is lower than that for hardened plants. A supplemental experiment showed that alfalfa plants in the full-bloom stage could be hardened to high temperatures, so that they were able to set pods at temperatures as high as 120°.

TABLE 1.—*Effect of air temperature and relative humidity on percentage of alfalfa flowers setting pods*

| Relative humidity (percent) | Flowers setting pods at indicated temperature (°F.) [1] | | | | | | | |
	60	70	80	90	100	110	120	Average (60–110)
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
10	35	51	62	51	45	15	0	43
30	46	51	52	46	50	17	0	44
50	42	44	56	48	47	13	0	42
70	41	40	40	37	34	6	0	33
90	42	37	38	42	32	6	0	33
Average	41	45	50	45	42	11	0	

[1] Differences greater than 8.4 among the percentages, after arc sine transformation, are significant at 5-percent probability.

The data indicate that the greatest influence of humidity on the number of pods produced was exerted at a temperature of 80° F. At 110°, 6 percent of the flowers set pods under a relative humidity of 90 percent and showed a gradual increase to 17 percent as the humidity decreased. At temperatures from 70° to 110°, similar increases were

noted in the number of flowers setting pods as the humidity decreased. The average values show a decrease in pod setting at temperatures above and below 80°, with a marked decrease above 100°.

No significant differences were obtained in the average percentage of pods set at temperatures from 60° to 100° F.; but the decrease in pod setting was highly significant ($P < 0.001$) at 110°, and no pods were formed at 120°. The differences in the average number of pods set at relative humidities from 10 to 50 percent were not significant, but the reduction from the number set at the lower humidities (10 to 50 percent) to the number set at the higher humidities (70 to 90 percent) was highly significant ($P < 0.001$).

No significant differences were observed in the average number of seeds per pod at the different humidity levels or at the different temperatures from 60° to 100° F. (table 2). A highly significant reduction in seeds per pod occurred at 110° ($P < 0.001$), and, as previously noted, no pods formed at 120°.

TABLE 2.—*Effect of air temperature and relative humidity on the number of alfalfa seeds per pod*

Relative humidity (percent)	Seeds per pod at indicated temperature (°F.) [1]							
	60	70	80	90	100	110	120	Average (60–110)
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
10	1.8	1.8	2.1	2.0	2.1	0.8	0	1.77
30	2.3	2.1	2.1	1.4	1.8	1.0	0	1.78
50	2.0	1.7	2.0	2.2	2.1	.8	0	1.79
70	1.8	1.9	1.9	1.8	1.6	.7	0	1.61
90	1.6	1.7	1.8	1.9	1.8	.6	0	1.57
Average	1.9	1.8	2.0	1.9	1.9	.8	0	

[1] A difference of 0.83 in number of seeds per pod is significant at 5-percent probability.

INFLUENCE OF SOIL MOISTURE AND ORGANIC RESERVES

The response of alfalfa plants to the four combined treatments of soil moisture and organic reserves is illustrated in figure 1. In each treatment 25 pots were used, and as many as 19,000 flowers were counted in the higher seed-setting groups. Table 3 presents the data obtained, expressed as 4-year averages of the number of racemes,

TABLE 3.—*Effect of organic reserves and soil moisture on seed production in alfalfa*

Item	Average under conditions of—			
	Low moisture, low reserves	High moisture, low reserves	Low moisture, high reserves	High moisture, high reserves
	Number	*Number*	*Number*	*Number*
Racemes per plant	13	31	32	66
Flowers per plant	151	437	511	1,068
Pods per plant	87	247	271	550
Seeds per plant	174	526	568	1,101
Flowers per raceme	12	14	16	16
Pods per raceme	7	8	9	8
Seeds per raceme	13	17	18	17
Seeds per flower	1.2	1.2	1.1	1.0
Flowers per pod	1.7	1.8	1.9	1.9
Seeds per pod	2.0	2.1	2.1	2.0

flowers, pods, and seeds per plant and their ratios to one another. Each annual replication was made with the same soil mixture and with cuttings from the same plant.
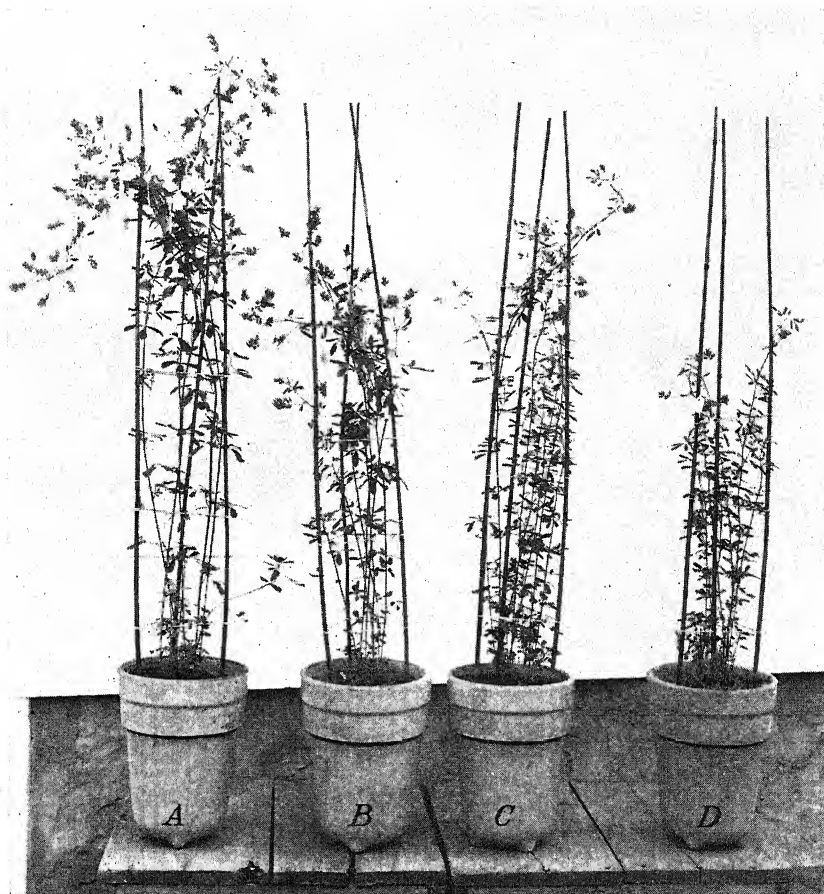
FIGURE 1.—Effect of soil moisture and organic reserves on setting of alfalfa seed: *A*, High moisture, high reserves; *B*, low moisture, high reserves; *C*, high moisture, low reserves; *D*, low moisture, low reserves.

Under conditions of low moisture and low reserves, alfalfa plants produced an average of 13 racemes; whereas, under conditions of high moisture and high reserves, the plants produced an average of 66 racemes. There was a corresponding difference in the number of flowers, pods, and seeds formed. The number of racemes produced under high moisture and low reserves was approximately the same as the number produced under low moisture and high reserves.

The relative importance of soil moisture and food reserves in the production of alfalfa seed under the four conditions is shown in table 4. Under the low-moisture condition, the high-reserve plants produced 146 percent more racemes per plant than were produced by the low-reserve plants. With high moisture, the increase due to higher

reserves was only 113 percent. Similar increases due to high reserves were obtained in the number of flowers, pods, and seeds produced. High moisture produced the same trend under the conditions of low and high reserves. With low reserves, the high moisture increased the production of racemes 138 percent over the low moisture; and with high reserves, the high moisture increased the number of racemes 106 percent.

TABLE 4.—*Effect of soil moisture and organic reserves on increase of alfalfa racemes, flowers, pods, and seeds per plant*

Item	Increase, due to high reserves, over low reserves at—		Increase, due to high soil moisture, over low soil moisture at—	
	Low moisture	High moisture	Low reserves	High reserves
	Percent	*Percent*	*Percent*	*Percent*
Racemes	146	113	138	106
Flowers	238	144	189	109
Pods	212	123	184	103
Seeds	226	109	202	94

Root samples were taken for carbohydrate and nitrogen analyses to determine the actual amount of organic reserves in the roots, and soil samples were also taken to determine the percentage of soil moisture maintained. The data in table 5 show that, on the date the seed crop started to develop, the low-reserve plants contained less total carbohydrates than the high-reserve plants. This condition existed until the last sampling date. By that time the low-reserve plants were able to store carbohydrates and build up a reserve. The low-reserve plants in 1942 were much lower in total carbohydrates at the first sampling date than the corresponding plants in 1941, because of the severer treatment they received before they were sampled.

TABLE 5.—*Percentage of soil water and of organic reserves in roots taken from pot samples*

Top growth development	Moisture	Reserves	Soil water	Total carbohydrates		Total nitrogen	
				1941	1942	1941	1942
			Percent	*Percent*	*Percent*	*Percent*	*Percent*
None	Low	Low	12.6	42.2	26.6	1.2	3.0
	High	....do	21.9	38.4	19.4	1.1	3.3
	Low	High	12.4	49.8	41.9	2.0	3.0
	High	....do	22.1	45.1	41.0	2.1	3.4
8 inches	Low	Low	12.0	43.8	22.5	1.5	3.1
	High	....do	18.5	45.1	20.0	1.3	3.4
	Low	High	11.7	50.9	37.5	1.7	3.1
	High	....do	20.0	50.0	35.4	1.7	3.2
Seed stage	Low	Low		42.2	36.4	1.2	3.4
	High	....do		52.3	37.2	1.8	
	Low	High		53.9	37.3	2.1	3.0
	High	....do		50.0	38.0	2.4	2.7

The average soil-moisture content of the low-moisture pots was 12.2 percent and that of the high-moisture pots 20.6 percent (table 5); these values are very near the desired percentages of 12 and 22, respectively.

The data on the effect of amount and time of irrigation on seed production are reported in table 6. The 2-year averages show little difference in the number of seed produced under the different treatments, except in the low-moisture pots. The plants given the low-high moisture treatment produced an average of 81 racemes per plant, which was 9 less than that produced by the continuous high-moisture treatment. There was a corresponding difference in the number of flowers, pods, and seeds formed. The ratio of flowers to racemes in the low-high pots was 15 to 1, which was the highest ratio for any of the treatments. Although the differences were small for the three treatments, it was evident that a low-moisture condition for a part of the time during the growth of the seed crop is not detrimental to seed production.

TABLE 6.—*Effect of amount and time of application of irrigation water on alfalfa seed production*

Item	Average under indicated treatment			
	Low moisture	Low-high moisture [1]	High moisture	High-low-high moisture [2]
	Number	*Number*	*Number*	*Number*
Racemes per plant	41	81	90	85
Flowers per plant	545	1,235	1,247	1,179
Pods per plant	230	526	547	510
Seeds per plant	385	868	866	770
Flowers per raceme	13	15	14	14
Pods per raceme	6	6	6	6
Seeds per raceme	9	11	10	9
Seeds per pod	1.7	1.7	1.6	1.5

[1] Low moisture from start of seed-crop growth until prebud stage, and high moisture during flowering and ripening period.
[2] High moisture until top growth was well started, low moisture until prebud stage, and high moisture until seed matured.

DISCUSSION

Previous investigations have shown the importance of the relation between the organic reserves in the roots and the vigor of the alfalfa plant. In the investigation reported here, it is shown that organic reserves stored in the roots are important also in relation to the production of seed. Air temperature, relative humidity, and soil moisture all affect the growth of the plant and thereby affect the storage of organic reserves.

The data from experiments in which temperature and humidity were controlled show the direct effect of these factors on the functioning of the reproductive parts of the plants. A statistical analysis of the data given in table 1 shows significant differences in the percentage of flowers setting pods as affected by temperature and humidity. The most favorable temperature was 80° F., and humidities above 50 percent were definitely detrimental to seed production. These data also indicate that the high humidity is more detrimental at 110° than at lower temperatures.

Soil moisture has a marked effect on the nature and rapidity of growth, thereby affecting the manufacture and storage of organic reserves. Any factor that influences the vigor and type of plant growth, thereby influencing plant-food manufacture and storage, may have a direct effect on seed production.

The data presented in table 5 show that in the low-moisture plants the values for total carbohydrates were generally slightly higher than those in comparable high-moisture plants, indicating that low moisture was conducive to a slower rate of top growth and thus enabled the plants to manufacture food faster than it was being used. This condition existed until the seed stage, when the top growth was of sufficient quantity and maturity in all treatments to build reserves. This development brought about the conditions described by Nightingale and Farnham (*10*), who stated that carbohydrate synthesis is important in obtaining ideal reproductive growth in sweet peas. In the author's experiments, less vigorously growing alfalfa, with high reserves, produced more racemes, flowers, pods, and seeds than plants with low reserves under comparable soil-moisture conditions (table 3). In the plants forced to grow under stress caused by external factors, a physiological situation was established favoring seed production when organic reserves were plentiful. These data show also that increased soil moisture had its greatest effect on plants under the less favorable condition of low reserves. The combination of high reserves and high soil moisture showed the highest production of seed in this experiment. However, preliminary tests indicate that this combination may not give the same results under field conditions where the plants are closely spaced, as the excess top growth and crowding would appear to retard blooming. In this greenhouse experiment each plant had a restricted space for root growth but adequate space for the maximum light required for branching and flowering.

Data for amount and time of irrigation (table 6) indicate that the low-high-moisture treatment brought about a condition during the early growth period favorable for the conservation of carbohydrates, and the delayed application of water made it possible for the energy stored in the form of carbohydrates to be used for seed production. This treatment produced the highest number of flowers and seeds per raceme. While the differences were not great, they were produced on a smaller number of racemes, indicating that the low-high-moisture treatment was less favorable for the production of racemes but more favorable for pollen germination and seed development.

SUMMARY AND CONCLUSIONS

A study has been made to determine the extent to which organic reserves in the roots, soil moisture, and climatic factors affect seed production in alfalfa.

Under the conditions of this experiment the number of alfalfa flowers setting pods decreased at temperatures above 100° F. The optimum temperature for pod setting was 80°.

The number of flowers setting pods increased as the relative humidity decreased from 90 to 10 percent. At 80° F., the difference between the percentage of pods set at 10 percent relative humidity and the percentage set at 90 percent was 24.

High reserves increased seed production; the greatest increase occurred when moisture was low. When the reserves were low, seed production was greater when the soil moisture was high.

Moderate air temperature, low humidity, and soil moisture below optimum produced the type of vegetative growth of alfalfa plants that

was conducive to the storage of high organic reserves, resulting in a physiological condition favorable to seed setting.

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RELATION OF MACROFUNGI AND MICRO-ORGANISMS OF SOILS TO DAMPING-OFF OF BROADLEAF SEEDLINGS[1]

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INTRODUCTION

At present the only reliable method of determining the damping-off potentialities of untried nursery soils is to ascertain the damping-off of susceptible seedlings in greenhouse or field trials. The object of the study reported here was to determine whether there is any relation between the abundance of macrofungi and micro-organisms in the soil and the damping-off of broadleaf seedlings.

MATERIALS AND PROCEDURE

In the first part of this study the macrofungi growing on Marshall silt loam in field and greenhouse tests were identified and the associated damping-off losses were compared for such broadleaf seedlings as *Ulmus americana* L., *U. pumila* L., *Robinia pseudoacacia* L., and *Chilopsis linearis* (Cav.) Sweet.

Dilution-plate counts were made to determine the number of fungi and bacteria in sandy loam and silt loam. The micro-organisms were studied also by the use of modified Cholodny soil slides. The resulting data were compared with the damping-off losses occurring on the same soils in crocks kept in the greenhouse. These tests were made at Lincoln, Nebr., from 1937 to 1939.

MACROFUNGI AND DAMPING-OFF

It has been observed in both field and greenhouse tests that the sporophores of certain fungi commonly follow specific agronomic crops.

One of the commonest sporophores found was that of the bird's-nest fungus, *Cyathus vernicosus* DC. It occurred most frequently in soil where corn had been grown or where wheat had followed corn. The base of the sporophore was generally attached to a piece of buried cornstalk. These sporophores have been observed only in the fall after rather heavy rains. In uncultivated fields they sometimes occur also in the spring, but the indications are that cultivation disturbs the soil to such an extent that it interferes with sporophore formation. What part, if any, this fungus plays in damping-off is

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unknown. However, greenhouse tests have indicated that soils on which the sporophores of *C. vernicosus* occurred were favorable to the broadleaf seedlings tested, since damping-off losses in these soils were relatively light.

Sporophores of *Naucoria semiorbicularis* Fr. have often been observed in fields where gramineous crops have been grown in successive rotations. They were particularly abundant in soils where wheat followed wheat or where rye followed wheat. On rye plots at the Nebraska State Agricultural Farm, these sporophores were observed to follow each drill row. Examinations showed that rhizomorphs extending from the sporophores were frequently attached to the bases of the plants or to their roots. However, Sprague (*12*)[3] found that closely related agarics were not pathogenic to cereals. Kauffman (*8*) also indicated that *N. semiorbicularis* is not pathogenic to grasses. Greenhouse tests showed that damping-off losses were heavy on such soils, and the abundance of these sporophores indicates that the soil is unfavorable for susceptible broadleaf seedlings.

Species of *Coprinus* observed both in the field and in potted greenhouse soils were especially common following alfalfa. Damping-off losses on such soils were heavy. The abundance of *Coprinus* sporophores indicates that such soils favor damping-off of broadleaf seedlings.

SOIL MICRO-ORGANISMS AND DAMPING-OFF

It was early recognized by pathologists that meteorological factors and environmental conditions have a strong influence on damping-off (*2*). They likewise affect the development of the soil micro-organisms (*11*). The number of micro-organisms in a soil is known also to be directly related to the depth, texture, and moisture content of the soil, the amount of organic matter present, the acidity, etc. (*14*). With so many complicating factors involved, it was recognized at the beginning of this investigation that in the time available a complete study of soil micro-organisms in relation to damping-off could not be made.

Probably the best method of determining the damping-off fungi present in the soil is by direct isolation from diseased seedlings and reinoculation to prove pathogenicity. In a previous paper (*17*) it was shown that *Rhizoctonia solani* Kühn and *Pythium ultimum* Trow are the principal fungi causing damping-off of broadleaf seedlings in Great Plains nurseries. Other micro-organisms in the soil may also be important.

DILUTION-PLATE STUDIES

METHOD

In preparing soils for dilution-plate studies, moisture contents were first determined on an oven-dry basis. After these differences had been taken into consideration, equivalent 10-gm. samples were placed in 100-cc. sterile water blanks and shaken vigorously for 2 minutes. Dilutions of 1:1,000 in peptone-glucose agar were used for the fungi. Bacteria were cultured on sodium albuminate agar at a dilution of 1:10,000 (*6*).

Dilution plates were poured in quadruplicate, and final counts of fungi were made after 4 days' incubation at 25° C., and of bacteria after 7 days' incubation.

[3] Italic numbers in parentheses refer to Literature Cited, p. 140.

<center>RESULTS</center>

These studies were repeated several times in conjunction with greenhouse damping-off tests, but a consistent relation has never been demonstrated between damping-off losses of broadleaf seedlings and the number of fungi and bacteria present in dilution plates. In general it appeared that the greater the number of fungi in the soil the heavier was the damping-off, whereas the reverse was generally true for bacteria except in soils occupied by legumes. It seemed possible that species of fungi present in the dilution plates might be important, but studies to obtain information on this point have also given negative results. Admittedly, studies to determine the relation of damping-off to the number of micro-organisms in the soil should be carried on for several successive years; but such studies were attempted only in a preliminary way in the present investigation. Whether a more complete study would be of greater practical value remains to be determined.

The kinds of fungi commonly appearing in the dilution plates were as follows: A yellow *Aspergillus* sp., *Trichoderma lignorum* (Tode) Harz, *Fusarium* spp. (both pink and white forms), *Rhizopus* spp., *Pythium* spp., *Mucor*, certain unidentified species of the Phycomycetes, and an unidentified white fungus. Of all these fungi, the yellow *Aspergillus* seemed to be the most prevalent. This fungus gave the medium adjacent to its hyphae a yellowish color.

No relation was found between the abundance of *Trichoderma* and damping-off losses, as reported by Weindling and Fawcett (*15*) for citrus seedlings. However, this is not regarded as important negative evidence, since the present investigations were made under conditions entirely different from those reported by Weindling and Fawcett. *Trichoderma* was much less abundant in these prairie soils than the other fungi named in the preceding paragraph.

While dilution-plate counts were used to determine the more common soil micro-organisms, such as the molds and bacteria, the microslide technique originated by Cholodny (*3*) was also employed to determine the micro-organisms in localized portions of the soil. Both methods have serious drawbacks. In dilution plates an approximation of the numbers of spore-forming fungi present in the soil can be obtained, but important non-spore-forming fungi, such as *Rhizoctonia*, are rarely isolated by this method. Thus dilution-plate counts may actually prove to be misleading for some purposes. The Cholodny method was used to identify the micro-organisms of the soil adjacent to plant roots, but the method cannot be used quantitatively. However, a modified Cholodny technique proved somewhat more useful.

<center>MODIFIED CHOLODNY SOIL-SLIDE STUDIES</center>

<center>METHOD</center>

In the present study, sterilized glass microslides were placed in sterile Petri dishes and coated with malt agar.[4] As soon as the agar had hardened, the slides were removed aseptically and slowly dried on an electric plate at 45° to 50° C. until the agar was reduced to paper thinness. These dried agar slides were inserted endwise into potted soils containing seedlings from which damping-off counts were

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[4] Malt agar was found to be especially desirable for general tests, principally because it adheres very firmly to the slide after drying.

being obtained in the greenhouse.[5] First a hole about 2 inches deep
was made by inserting a spatula into the soil and pulling it backward
so that one face of the hole remained smooth and flat. Then the
dried agar surface was placed against the flat side of the hole, and
the loosened soil was packed down to hold the slide firmly in place.

These slides were left in the soil for 3 to 4 days. During this
time the dried malt agar absorbed enough moisture from the soil to
become softened, and thus furnished a suitable substratum for the
growth of micro-organisms. Finally, the slides were extracted from
the soil by pulling the agar-coated surface away from the flat side of
the hole. After removal, the slides, with the agar surface up, were
placed in Petri dishes and brought to the laboratory, where they were
examined with a binocular microscope. In this way it was sometimes
possible to detect individual colonies of fungi or bacteria, from which
isolations were made for identification purposes. Ultimately the
slides were slowly heated until the agar was again dried to paper
thinness; then they were stained for detailed examinations. Best
results were obtained by staining the dried agar slides with a rose
bengale solution as described by Conn (5). After staining, the slides
were ready for observation under high-power magnification. These
stained, dried agar slides have been kept for several years, and they
are still good for microscopic study even with an oil-immersion lens.

RESULTS

In slides observed under a binocular microscope before drying, it
was sometimes possible to detect the movements of nematodes and
an occasional amoeba on the surface of the agar. In some cases the
nematodes seemed to be feeding on the fungi, particularly on the
fungus hyphae.

After examining dozens of stained, dried agar slides, the writer
found no evidence of antagonism between fungi, between bacteria, or
between fungi and bacteria, although actinomycetes appeared to
develop best in less densely populated areas of the slides. As Conn
(5) had found, bacteria were most abundant in soils of high moisture
content. The species of *Aspergillus* that caused a yellowish discolora-
tion in the agar dilution plates was also frequently observed on the
dried agar slides.

In soils with abundant nematodes, there appeared to be a relation
between the number of nemas on the dried agar slides and the kind of
rotation crop. In these tests, nematodes were found to be more
numerous in soils obtained from cereal plots than in those from legu-
minous rotations. Other tests had already indicated that the use of
most legumes as preceding crops increased damping-off of broadleaf
seedlings. The relation of damping-off to the number of nematodes
is interesting, since Christie and Arndt (4) were first inclined to believe
that nemas supposed to be saprophytic may cause root troubles of
cotton seedlings. They later concluded (1) that their experiments
failed to prove that the species of nematodes investigated were factors
of primary importance in the etiology of damping-off, or sore shin,
in cotton. However, Wilde (16) considered that nematodes were
directly connected with damping-off losses in coniferous nurseries in

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[5] The modified Cholodny slide method, which was developed during the course of these studies, was later
found to be almost identical with that previously described by Kriuchkova (9).

Wisconsin. It is apparent that the observations presented here, which indicated that soils with numerous nematodes have less damping-off than soils with smaller populations, need confirmation.

Figures 1 and 2 show photomicrographs of stained, dried malt-agar slides, which illustrate some of the more common micro-organisms observed by the use of the modified Cholodny technique.

Pythium, other unidentified species of Phycomycetes, *Rhizoctonia*, *Fusarium*, *Penicillium*, and many other fungi were readily identified on these stained, dried agar slides.

Likewise of interest was the fact that the modified Cholodny slides showed clearly the initial stage of sclerotial formation of *Rhizoctonia*. It appeared that where *Rhizoctonia* hyphae came in contact with a solid object, such as the glass slide, they flattened out considerably prior to sclerotial formation.

Since this modified Cholodny technique satisfactorily showed the presence of bacteria and actinomycetes, as well as spore-forming and non-spore-forming fungi, besides some forms of microfauna, it is regarded as a valuable supplement to soil-dilution studies in determining the nature of the microbial population of the soil.

The Cholodny technique is applicable to both field and greenhouse conditions. By using selective agar it is possible in some instances to study the development of specific organisms while nearly excluding others. However, if specialized agar is used, preliminary tests indicate that malt sugar should be included in the medium, since this carbohydrate when heated has a marked tendency to cause the agar to stick firmly to the slide.

A method developed by Canadian workers (*7, 13*) has enabled them to determine the micro-organisms present within the roots of certain plants, but it is not regarded as applicable to the present study. No attempt was made to make comparative tests by direct microscopic examination of the soil as described by Kubiena and Renn (*10*).

DISCUSSION

From the foregoing preliminary results, it does not appear likely that a thorough and more complete study of the microbial population of the soil would furnish a satisfactory clue to the damping-off potentialities of untried nursery sites. In such studies it would be necessary to include many soil types as well as different crop sequences, and the tests should extend over several years so that the effects of meteorological factors could be determined. Such studies are complicated by the fact that climatic variations affect the host, the activity of the microbial population of the soil, and, over a period of years, the soil itself. Whether these effects run parallel, run in opposite directions, or have any relation at all is at present not definitely known. In the final analysis, standards should be established on the basis of definite numbers of micro-organisms and should not be merely comparative. Assuming that such a standard could be obtained, it is doubtful whether it would be of much value to field technicians, since the technique required to make it dependable would probably be so refined as to preclude practical use.

At the present time it appears that the best method of predetermining the damping-off hazard of an untried soil is by greenhouse tests. However, microbiological studies are of considerable scientific interest
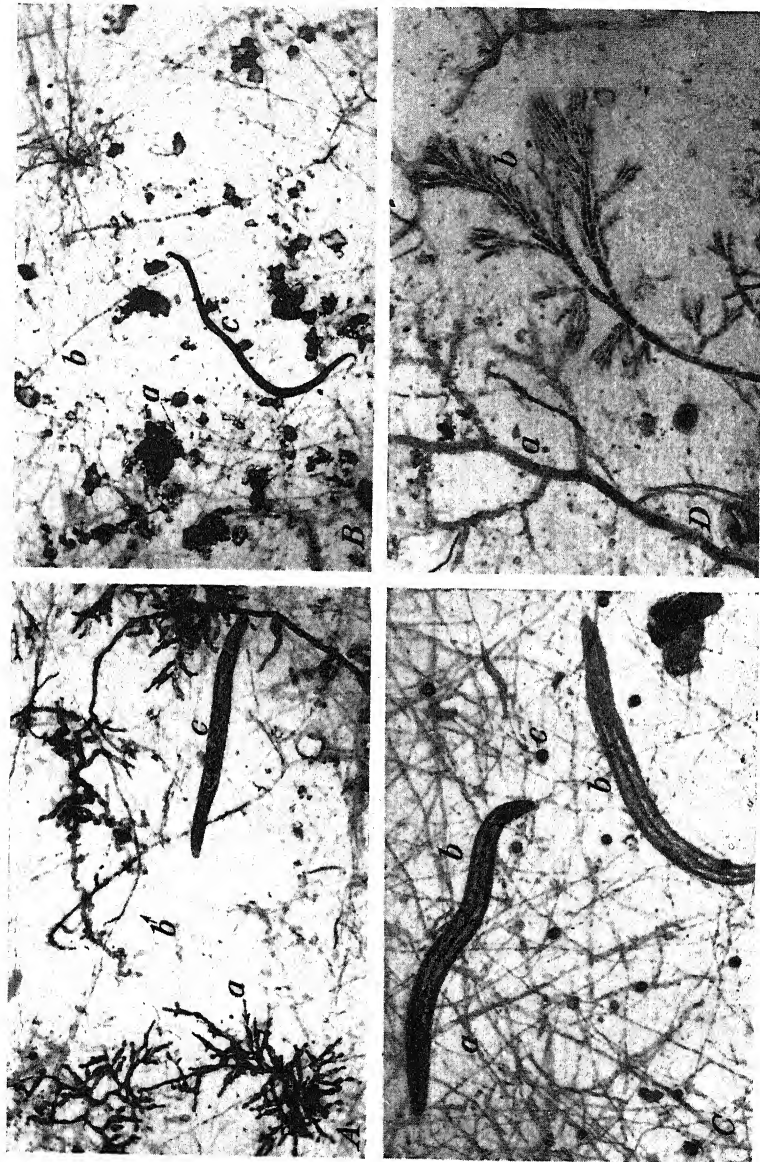
FIGURE 1.—Dried agar-soil slides stained with rose bengale (modified Cholodny slide technique). A: a, Beginning of sclerotial formation of *Rhizoctonia solani*; b, hyphae of other fungi; c, a nematode. ×118. B: a, Soil particles; b, *Actinomyces*, hyphae; c, a nematode. ×118. C: a, Hyphae of various soil fungi: b, nematodes; c, *Pythium* sp., oospore. ×233. D: a, *Phycomycete*, hyphae; b, *Penicillium*. ×233.
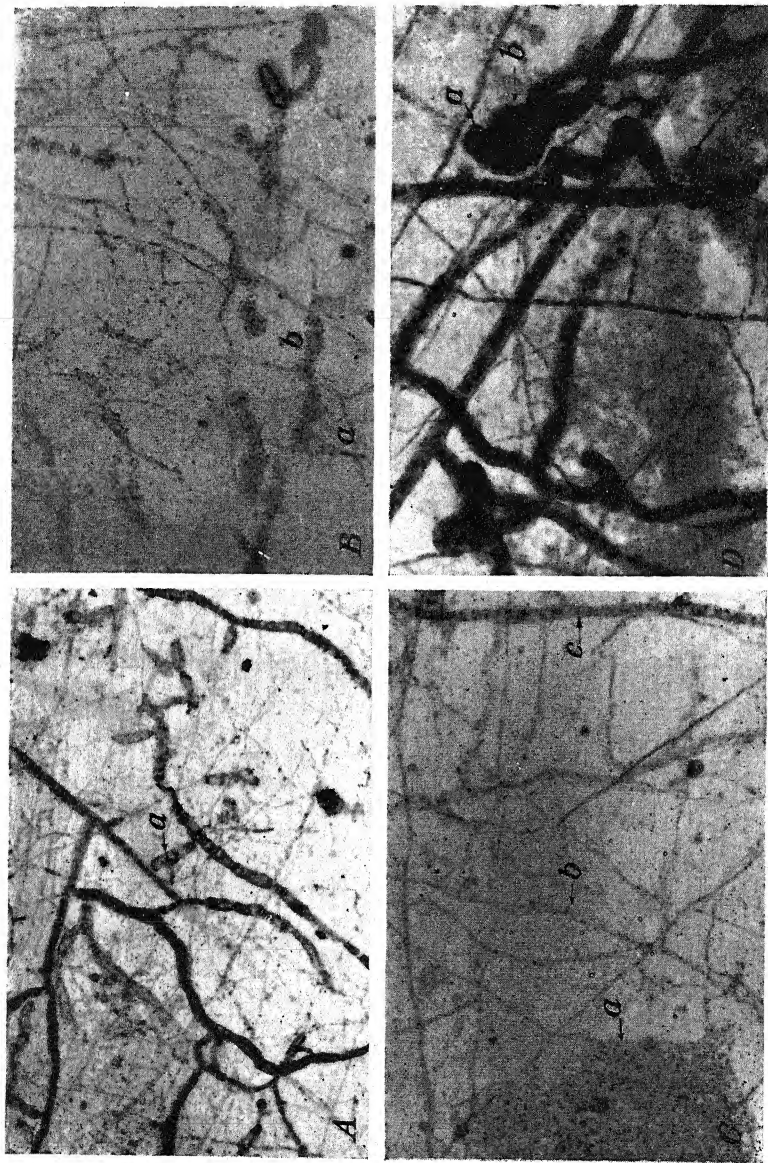
FIGURE 2.—Dried agar-soil slides stained with rose bengale (modified Cholodny slide technique). A: a, *Fusarium* sp.: a, Hyphae above other mycelial strands. ×233. B, *Actinomyces*: a, Hyphae; b, spores. ×233. C: a, Bacterial colony; b, *Actinomyces*, hyphae; c, *Fusarium*, hyphae. ×560 D, *Pythium* sp., hyphae showing (a) antheridium and (b) oogonium. ×560.

if not of practical value, and in special cases where numerous treatments are being tested on the same soil such studies may prove useful in determining variations between individual plots or treatments.

SUMMARY

Observations on the occurrence of soil macrofungi were made, and an apparent relation was noted between the occurrence of such fungi and the damping-off of broadleaf seedlings. The occurrence of sporophores of *Cyathus vernicosus* was associated with soils favorable to the growth of broadleaf species and low incidence of damping-off. An abundance of sporophores of *Naucoria semiorbicularis* and *Coprinus* spp., on the other hand, was associated with soils in which damping-off was high.

Soil micro-organisms were studied by dilution-plate counts and by a modification of the Cholodny microslide technique. The results obtained by the former method were not consistent. The general trend indicated that the greater the number of fungi present in the soil the heavier was the damping-off loss. However, soils containing the largest number of bacteria generally showed the least damping-off, except for sites on which leguminous crops preceded the broadleaf seedlings.

In a modified Cholodny technique, dried agar-coated slides were inserted into the soil to furnish a medium for sporulating and non-sporulating fungi, bacteria, and other micro-organisms. Nematodes were frequently observed in abundance; these appeared to be most numerous in soils on which cereal crops had been grown and on which damping-off losses were relatively light. The modified Cholodny slide technique used is believed to be of value for making a detailed study of the microbial population of the soil.

On the basis of the data presented, it does not appear likely that detailed microbiological studies of the soil will prove of practical value in predicting damping-off losses.

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EFFECT OF POTASSIUM DEFICIENCY AND OF POTASSIUM DERIVED FROM DIFFERENT SOURCES ON THE COMPOSITION OF THE JUICE OF VALENCIA ORANGES [1]

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INTRODUCTION

The work reported herein was undertaken for the purpose of determining the progressive changes in some of the biochemical characteristics of the juice of orange (*Citrus sinensis* (L.) Osbeck) when the fruit was grown under controlled fertilization, the only variable nutritional factor being the amount and source of potash. This study constitutes one phase of an experimental project of the Bureau of Plant Industry, Soils, and Agricultural Engineering which has been in progress for 5 years at Orlando, Fla. The results of certain other phases of the experiment have already been reported (6).[3]

The specific factors considered in the present work concern the progressive changes in the composition of the juice, during the final 6 months of development of the fruit, with respect to sugars, ascorbic acid, buffer capacity, citric acid, ash and its alkalinity, and potassium, when the trees were fertilized with potash from different sources and applied at different levels. All other fertilizer ingredients were applied at a constant level. The application levels ranged as high as twice the customary levels.

Many Florida citrus growers hesitate to use potash from chloride sources in their fertilizer mixes, mainly because of the deleterious effect that fertilizers high in chlorine are known to have on such crops as tobacco and potatoes, although evidence of any harmful effects from the moderate use of chloride salts on citrus is lacking. Hume (28) stated:

Of all the materials [sources of potash] the sulfates are safest and best. There is a general impression that muriate of potash is not a good substance to use on orange trees, and while its deleterious effects have never been elucidated, the impression is probably well grounded.

Since the muriate salts of potash are less expensive per unit of potash than the sulfate or the sulfate of potash-magnesia, it is highly desirable from an economic point of view to ascertain whether there is any basis for the prejudice against their use.

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The nutritional value of orange juice, as pointed out by Jones (*29*), is probably due to three main properties, each measurable by chemical means. These are the vitamin C content, the alkalizing value, and the caloric value, the last depending mainly on the sugar content. The principal nutritional value of oranges lies in their vitamin C content. The alkalizing value is also considered to be important (*10*), but less stress seems to have been placed on the actual energy value of the fruit.

In recent years both growers and consumers have become more conscious of quality in citrus fruit. This factor has been strongly emphasized at meetings of leaders of the citrus industry in Florida, in which the need for a "medicinal" grade of citrus fruit has been discussed; i. e., a fruit with an exceptionally high content of ascorbic acid, ash, and sugar and one that can be specifically recommended for use by invalids and children. It is important, therefore, to know how far and in what manner potash contributes to the production of fruit possessing these characteristics.

REVIEW OF LITERATURE

Although the necessity of potassium for plant growth has long been recognized, its exact role in the development of plants is not known. As Miller (*33, p. 318*) has stated, " . . . its function is only surmised by noting the effects on the plant in its absence."

Much of the experimental work with potash has been carried on to determine its effect on yield of the crop. Such investigations on citrus fruits have shown that yields can be increased by the inclusion of potash in the fertilizer, but that the point of diminishing returns from potash applications varies, depending upon the soil type and its natural fertility with respect to this element (*6; 39; 44, pp. 44, 45; 45, p. 47*). This latter fact has probably influenced conclusions such as those reached by Young (*52*), who reported no effect of potash fertilization on the composition of the orange but stated that the soil used in his studies was naturally well supplied with potash.

Light soils, such as are found in Florida, lend themselves readily to fertilization experiments. In 1894, Webber (*50*), basing his fertilizer recommendations on observations of intelligent citrus growers, suggested that potash applications be decreased and sulfate of ammonia be used when it was desirable to sweeten the fruit and that potash application be increased if a more acid fruit were preferred.

Anderssen (*2*) stated that potassium does not influence the sugar content of oranges, but like Young he used a heavy soil. He correlated high potash fertilization with high juice acidity. Morris' (*35*) findings were similar under similar conditions. Benton and Stokes (*8*) reported that the total sugar content of orange juice was less in fruit from trees fertilized with large amounts of potash. Colby (*13*) found an inverse relation between potash fertilization and the concentration of sugar in orange juice.

The American Medical Association (*1, p. 365*) and Daniel and Munsell (*16*) listed oranges as among the richest sources of vitamin C. Roy and Bahrt (*42*) and Fudge and Fehmerling (*19*) showed that a relation exists between the vitamin C content of oranges and various soil fertility factors. Nelson (*36*) found that repeated spraying with arsenicals caused a decrease in the vitamin C content of oranges.

Kinman (*30*) reported that the flavor of oranges grown without potash was insipid. This statement could be interpreted to mean that the citric acid content was less than that of well-fertilized fruit of the same age. Hilgeman et al. (*27*) found that when potash was withheld for 3 years from grapefruit trees, the fruit juice contained less acid than that of fruit produced on well-fertilized plots.

McKaig and Hürst (*31*) found that application of potassium increased the pH values, the ash, and the potassium content of sugarcane, but Hartt (*24*) could establish no differences in pH or in titratable acid attributable to the element. She found that the amount of potassium in the ash of sugarcane was directly proportional to the amount of the element supplied in the nutrient solution. In her studies potassium was the only element that showed any strong tendency to migrate.

Haas (*20*) could find no evidence of any relation between the potassium in the ash of orange juice and that in the soil. The amount of potassium in the whole fruit, however, was increased by soil fertilization with potash.

Both the citric acid content (*14, 18, 22*) and the ascorbic acid content (*18, 21, 51*) of citrus fruit juices have been shown to decrease periodically during the maturing process. The sugar content, on the other hand, progressively increases. Collison (*14*) reported that the concentration of sucrose increases at a faster rate than does that of the reducing sugars. Harding et al. (*22*) noted the same seasonal trend, while Braverman and Carmi (*12, p. 152*) reported that sucrose increased but that the reducing sugars remained constant in the juice throughout the season. The last-named workers based their conclusions on analyses of Palestine oranges. Hilgeman and Smith (*26*) called attention to the fact that grapefruit, when mature, contain a maximum amount of sucrose.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The experiment was performed on a block of uniform bearing Valencia orange trees set out in 1924. This block is located about 1 mile east of Narcoossee, Fla. The trees were budded on rough lemon rootstocks. The data reported in this paper were derived from fruit of the 1939–40 crop after the fertilizer applications had been continued for 5 to 5½ years.

The soil type was Norfolk fine sand, a soil widely planted to citrus throughout the State and adaptable to experimentation because of its permeability and low mineral and organic-matter content.[4] As the location was exposed to 55 or 60 inches of rainfall, there was a minimum of residue of soil nutrients from previous cultural treatments.

The soil reaction was fairly uniform throughout the grove, ranging from pH 4.5 to 5.5. A soil of this type containing little organic matter or colloidal material readily reflects by its pH values the presence of residual substances from previous fertilizer applications. Moreover the pH values of such soils can easily be altered in a rather short time. Although citrus trees grow in soils covering a wide range of pH values

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[4] Peech (*37*) reported the following figures from analyses of 50 samples of Norfolk fine sand: Exchange capacity, 1.67 to 5.00 milliequivalents; organic matter, 0.93 to 3.10 percent.

and the opinions of growers differ widely as to which is best for the growth of citrus trees, a pH value of approximately 6.0 is usually considered the most desirable.

The fertilizers used were nearly neutral in reaction (residual effect), and there was little or no change in the pH of the soils in the experimental plots during the course of the experiment.

Valencia orange trees were selected for the experiment because this variety is the predominant late orange grown in the State. It might be mentioned that rough lemon rootstock is particularly adapted to well-drained light sandy soil.

Each experimental plot consisted of five trees in a row, with the exception of plots 27 and 29, which contained seven trees each. Each

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O   O   O   O   O   O   O   O   O   O   O   O   O   O   O
O   5X  O   6X  O   7X  O   8X  O   9X  O   10X O   11X O
O   5X  O   6X  O   7X  O   8X  O   9X  O   10X O   11X O
O   5X  O   6X  O   7X  O   8X  O   9X  O   10X O   11X O
O   5X  O   6X  O   7X  O   8X  O   9X  O   10X O   11X O
O   5X  O   6X  O   7X  O   8X  O   9X  O   10X O   11X O
O   O   O   O   O   O   O   O   O   O   O   O   O   O   O
O   12X O   13X O   14X O   15X O   16X O   17X O   18X O
O   12X O   13X O   14X O   15X O   16X O   17X O   18X O
O   12X O   13X O   14X O   15X O   16X O   17X O   18X O
O   12X O   13X O   14X O   15X O   16X O   17X O   18X O
O   12X O   13X O   14X O   15X O   16X O   17X O   18X O
O   O   O   O   O   O   O   O   O   O   O   O   O   O   O
O   19X O   20X O   21X O   22X O   23X O   24X O   25X O
O   19X O   20X O   21X O   22X O   23X O   24X O   25X O
O   19X O   20X O   21X O   22X O   23X O   24X O   25X O
O   19X O   20X O   21X O   22X O   23X O   24X O   25X O
O   19X O   20X O   21X O   22X O   23X O   24X O   25X O
O   O   O   O   O   O   O   O   O   O   O   O   O   O   O
O   27X 27X 27X 27X 27X 27X 27X O   26X 26X 26X 26X 26X O
O   O   O   O   O   O   O   O   O   O   O   O   O   O   O
O   29X 29X 29X 29X 29X 29X 29X O   28X 28X 28X 28X 28X O
O   O   O   O   O   O   O   O   O   O   O   O   O   O   O
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FIGURE 1.—Plan of experiment on Valencia oranges grown on rough lemon rootstocks, Narcoossee, Fla., 1935–40. X, Trees in experimental plots. O, Trees in buffer rows. The figures are plot numbers.

plot was separated from adjacent plots by a row of buffer trees (fig. 1). By thus isolating each treatment the effects of adjoining fertilizer applications were minimized. Each treatment was replicated three or four times at random.

FERTILIZER APPLICATIONS

The compositions and grades of the fertilizers used are shown in table 1. For the sake of brevity the treatments in which no potassium was used are termed the "no-potash" treatments; those which consisted of an annual application of 1.7 to 2.3 pounds [5] of potash per tree are called the "medium" muriate, sulfate, or sulfate of potash-magnesia treatments, while those in which 2.7 to 3.6 pounds of potash

[5] In the fall of 1934, a single application of 10 pounds per tree was made. During 1935 three applications of 10 pounds each per tree were made. The annual application per tree was increased by 2 pounds each succeeding year thereafter, so that in 1939, each tree received a total of 38 pounds of fertilizer.

per tree was applied annually are referred to as the "high" muriate, sulfate, or sulfate of potash-magnesia treatments.

TABLE 1.—*Composition and grades of fertilizers used in the experiment with Valencia oranges grown on rough lemon rootstocks, Narcoossee, Fla., 1935–40*

| Treatment | Potash applied per tree per year | Fertilizer [1] | | Plot numbers |
		When applied	Grade (N–P_2O_5–K_2O)	
	Pounds			
No potash	None	Spring	4–8–0	5, 12, 22, 26
		Summer	3–8–0	
		Fall	3–8–0	
Medium sulfate of potash	1.7 to 2.3	Spring	4–8–4	6, 16, 21, 24
		Summer	3–8–5	
		Fall	3–8–8	
Medium muriate of potash	1.7 to 2.3	Spring	4–8–4	9, 13, 25
		Summer	3–8–5	
		Fall	3–8–8	
Medium sulfate of potash-magnesia	1.7 to 2.3	Spring	4–8–4	7, 17, 19, 23
		Summer	3–8–5	
		Fall	3–8–8	
High sulfate of potash	2.7 to 3.6	Spring	4–8–4	10, 14, 20
		Summer	3–8–10	
		Fall	3–8–13	
High muriate of potash	2.7 to 3.6	Spring	4–8–4	8, 18, 28
		Summer	3–8–10	
		Fall	3–8–13	
High sulfate of potash-magnesia	2.7 to 3.6	Spring	4–8–4	11, 15, 29
		Summer	3–8–10	
		Fall	3–8–13	

[1] Potash source indicated in description of treatment; potash derived from 50 percent muriate of potash, 48 percent sulfate of potash, and 26 percent sulfate of potash-magnesia; phosphoric acid from superphosphate and steamed bonemeal; nitrogen, one-half from sodium nitrate and ammonium sulfate and one-half from steamed bonemeal, cottonseed meal, and tankage.

Fertilizer was applied three times a year; the spring application was made in March, the summer application in July, and the fall application in November or December. The mix was broadcast by hand evenly over the entire area defined by the treatment, from tree trunk to tree trunk, to insure an even distribution over the entire root-system area.

All fertilizers were formulated and mixed by or under the supervision of employees of the United States Department of Agriculture. The materials used were all of the market grade customarily applied.

Insect control was conducted by the owner of the grove, and all spray and dust applications were made uniformly throughout the grove. A volunteer cover crop was allowed to grow and was disked in the fall. All other cultural practices, such as cultivation and pruning, were carried on by the owner of the grove.

SAMPLING

The first samples were taken from the trees on September 16, 1939, when the fruit was of sufficient size to yield adequate amounts of juice for analysis. Thereafter samples were obtained at intervals of approximately 3 weeks [6] throughout the growing season until March 8, 1940, when the grove was picked commercially.

[6] An exception to this schedule occurred unavoidably between October 7 and November 4, a lapse of 4 weeks.

Forty oranges were picked from each plot, the procedure being to clip the fruit from the periphery of the tree at a height of about 5 feet.   Appleman and Richards (4) showed statistically that samples of oranges consisting of less than 20 to 30 fruits constitute inadequate representation of the supply when conclusions are to be derived from data obtained from such samples.   Harding et al. (22) demonstrated the adequacy of orange samples of 25 or more fruits as being fully representative of the supply.

No attempt was made to select fruit for size or color.   The only precaution observed was to avoid taking late-bloom fruit.

The fruit from each plot was placed in labeled bags and carried to the laboratory where it was carefully washed and surface-dried. Each fruit was cut in half, and the juice was lightly expressed by hand squeezing, care being taken to keep the sample as free from rind oil as possible.   The juice was then filtered through two thicknesses of clean cheesecloth and thoroughly mixed.

Samples were taken immediately for titratable acidity, ascorbic acid, and sugar determination.   About 250 ml. of the remaining juice was stored in stoppered Erlenmeyer flasks at 0° C. until further samples were needed for other determinations.   All determinations were completed in the 3-week intervals between samplings.   The sequence of making the analyses for the various constituents was based upon the susceptibility to change of the constituents, that is, the most labile substances such as ascorbic acid and sugars were determined at once, whereas, the citric acid, ash and its alkalinity, and buffer capacity were determined later in the intersampling period.

SUGARS

Reducing sugars were determined by the application of a modification of the Scales method (43) after first clarifying a weighed sample of the orange juice with neutral lead acetate.   Total sugars were determined by the use of the same method on a clarified sample of the juice which had been inverted in the presence of 0.6 N hydrochloric acid at 25° C. for 12 hours.   Nonreducing sugars were calculated from the difference between total and reducing sugars by utilizing appropriate conversion factors.   Reducing sugars were calculated as glucose and nonreducing as sucrose.

ASCORBIC ACID

Ascorbic acid was determined by titrating a 5-ml. sample of the juice in 10 ml. of 8 percent acetic acid with an aqueous solution of sodium 2,6-dichlorobenzenoneindophenol, which had been standardized against a solution of pure ascorbic acid.   The end point observed was a faint pink color, which persisted for 30 seconds.   The method was essentially that described by Bessey and King (9).   All solutions used were prepared immediately before use, as it has been found that ascorbic acid solutions slowly deteriorate on aging, and the redox dye yields a rather indefinite muddy end point after aging for 3 or 4 days.   The powdered dye was thoroughly washed with anhydrous ethyl ether before being dissolved in water, and its strength was adjusted so that a titration of 5 to 10 ml. was obtained by the use of a 5-ml. sample of orange juice.   As is customary, the ascorbic acid content of the juice is reported as milligrams per milliliter of juice.

CITRIC ACID

Citric acid was determined by precipitation from a sample of the juice with lead acetate and isolating the salt by centrifuging and washing. Lead was precipitated with hydrogen sulfide and removed by filtration. The citrate was oxidized and brominated to form penta-bromacetone, which was collected on a tared Gooch crucible, dried for 24 hours over anhydrous sulfuric acid in a vacuum desiccator, and weighed. The percentage of citric acid in the original juice was calculated from these data. The method is that described by the Association of Official Agricultural Chemists (*5*).

The titratable acidity (*6*) was determined by titrating a sample of the fresh juice with standard sodium hydroxide solution; phenolphthalein was used as the indicator. The acidity was calculated as percentage of citric acid.

BUFFER CURVES AND INDICES

Buffer curves were obtained by the following procedure. The pH of a 50-ml. sample of the juice was determined by means of the quinhydrone electrode. Increments of 3 ml. of 0.5 N hydrochloric acid were added. The pH of the solution was determined after each addition. This procedure was repeated until the pH of the solution was 1.0 or less. A new 50-ml. portion of the juice was then treated with increments of 3 ml. of 0.5 N sodium hydroxide, the pH being determined after each addition of base until a pH of 9.0 was attained.

From data so obtained curves were drawn with pH values as the abscissas and gram equivalents of acid or alkali as ordinates.

The same data were employed to plot the buffer indices as defined by Van Slyke (*49*), the graphs representing the slopes of the pH-titration curves $\frac{(dB)}{(dpH)}$ as ordinates and pH values as abscissas.

ASH AND ITS ALKALINITY

Ash was determined by weighing a 5-gm. sample of juice in a small porcelain crucible, drying it in an oven at 105° C. for 24 hours, and then ashing to constant weight at 400° to 500° in an electric muffle furnace.

The ash was dissolved in an excess of 0.1 N hydrochloric acid, gently heated to insure complete solution, cooled, and titrated with 0.1 N sodium hydroxide; methyl orange was used as the indicator. The alkalinity of the ash was reported as the number of milliliters of 0.1 N hydrochloric acid required to neutralize the ash from a 100-gm. sample (*5*).

POTASSIUM

Potassium was determined in the sample used for ash and alkalinity of the ash. It was determined as the chloroplatinate, as described by the Association of Official Agricultural Chemists (*5*).

STATISTICAL TREATMENT

The data obtained were analyzed statistically according to methods outlined by Fisher (*17*) and Snedecor (*48*). Analysis of variance was applied to determine the significance of differences both between seasonal means of treatments and between treatments for the last sampling on March 8, 1940, when the fruit was harvested commercially.

Data from the last sampling were the only single sampling-date figures subjected to statistical analysis because, being from the harvest sample, they represented analyses of juice from fruit as actually bought by the consumer. Differences between means were considered significant when they attained or exceeded the 5-percent level, and highly significant at or above the 1-percent level.

Correlations were established by applying the method of least squares for regression curves. Correlation coefficients were determined by application of formulas given by the above-mentioned texts (*17, 48*).

RESULTS

SUGARS

Data showing the influence of potash on the reducing sugar, non-reducing sugar, and total sugar content of fruit from the seven treatments during the period of study are presented in table 2. The data are shown graphically in figure 2.

TABLE 2.—*Sugar content of Valencia orange juice on various dates during the maturation period, 1939–40*

[Means of plot replicates]

Sugar determined and potassium treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
Reducing sugars (as glucose):										
No potash	3.89	4.45	4.71	5.13	5.68	5.98	5.64	5.96	6.72	5.35
Medium sulfate of potash	3.14	3.61	3.72	4.01	4.61	4.70	4.73	5.09	5.61	4.36
Medium muriate of potash	3.04	3.40	3.55	3.78	4.43	4.45	4.40	4.80	5.27	4.12
Medium sulfate of potash-magnesia	3.07	3.55	3.71	3.87	4.51	4.64	4.69	4.89	5.42	4.26
High sulfate of potash	2.82	3.43	3.55	3.72	4.27	4.34	4.44	4.81	5.42	4.09
High muriate of potash	3.08	3.59	3.64	3.85	4.54	4.53	4.44	4.77	5.28	4.19
High sulfate of potash-magnesia	3.04	3.69	3.73	4.02	4.68	4.70	4.76	5.11	5.56	4.37
Difference required for significance:										
5-percent level									.500	.110
1-percent level									.759	.154
Nonreducing sugars (as sucrose):										
No potash	1.37	1.83	2.28	3.61	4.28	4.48	4.47	4.32	4.50	3.46
Medium sulfate of potash	1.31	1.83	2.46	3.75	4.55	4.69	4.84	4.62	4.67	3.64
Medium muriate of potash	1.34	1.76	2.25	3.74	4.38	4.51	4.86	4.67	4.79	3.59
Medium sulfate of potash-magnesia	1.28	1.66	2.26	3.68	4.46	4.72	4.74	4.80	4.97	3.62
High sulfate of potash	1.12	1.81	2.32	3.57	4.49	4.56	4.88	4.74	5.04	3.61
High muriate of potash	1.48	1.81	2.49	3.71	4.40	4.65	4.94	4.51	4.73	3.64
High sulfate of potash-magnesia	1.41	1.83	2.43	3.81	4.52	4.66	5.25	4.69	5.05	3.74
Difference required for significance:										
5-percent level									(1)	.147
1-percent level									(1)	.204
Total sugars:										
No potash	5.26	6.23	6.99	8.74	9.96	10.46	10.11	10.27	11.22	8.80
Medium sulfate of potash	4.45	5.43	6.18	7.92	9.16	9.39	9.57	9.71	10.28	8.01
Medium muriate of potash	4.33	5.15	5.80	7.52	8.81	8.98	9.25	9.47	10.06	7.71
Medium sulfate of potash-magnesia	4.35	5.21	5.97	7.55	8.97	9.36	9.43	9.69	10.38	7.88
High sulfate of potash	3.95	5.24	5.87	7.28	8.75	8.91	9.38	9.55	10.45	7.71
High muriate of potash	4.56	5.40	6.13	7.56	8.94	9.16	9.38	9.29	10.01	7.83
High sulfate of potash-magnesia	4.45	5.51	6.16	7.84	9.20	9.36	10.01	9.81	10.61	8.11
Difference required for significance:										
5-percent level									(1)	.180
1-percent level									(1)	.251

[1] Differences not significant.

In general all the sugars studied showed gradual but regular increases in orange juice from all treatments as the fruit ripened. The mean concentration of reducing sugar was found to be significantly higher in juice from the no-potash plots than in that from any of the potash-treated plots, when the season as a whole was considered.
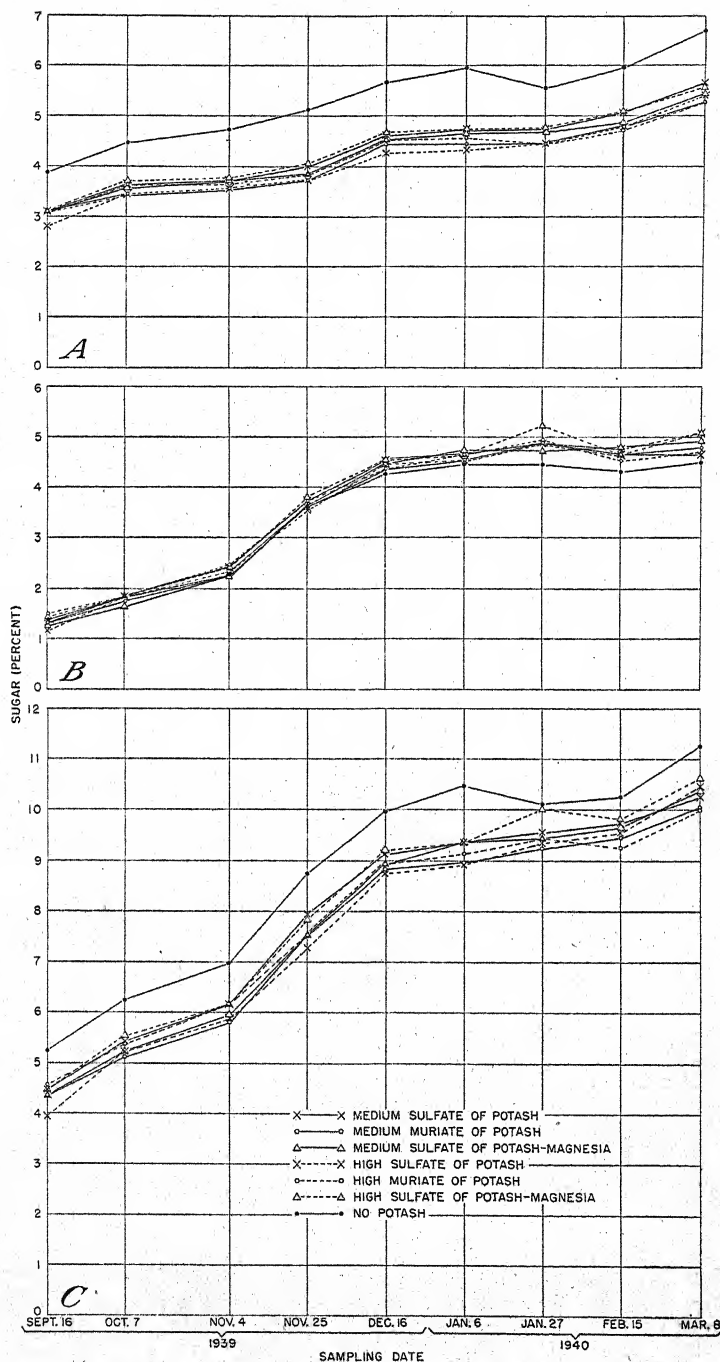
FIGURE 2.—Seasonal changes in the sugar content of Valencia oranges grown on rough lemon rootstocks, Narcoossee, Fla., 1939–40: *A*, Reducing sugars (glucose); *B*, nonreducing sugars (sucrose); *C*, total sugars.

Also a highly significant difference was found between the concentration of reducing sugar in the no-potash sample of juice and in that of samples from each of the potash-treated plots when the fruit was harvested on March 8. On that date the reducing sugar content of the various juices from the six potash-fed treatments was practically the same.

A larger amount of nonreducing sugars was found in the seasonal mean of juice from the high sulfate of potash-magnesia plot than in that of juice from the no-potash plots, the difference being highly significant. Smaller and less significant differences were observed in the seasonal means of other treatments when compared with that of the juice from the no-potash treatment. No significant differences were noted between any of the juices with respect to nonreducing sugars at the harvest sampling.

Highly significant differences existed between the seasonal mean for the total sugar content of fruit juice from the no-potash treatment and the means of the juices from each of the potash treatments, but the difference for the final harvest sample was not significant. The greater seasonal-mean concentration of sugar in juice from the no-potash plots was accounted for by the increase in the reducing sugar in those juices, since there was actually a slightly smaller sucrose content in juice from plots which were deprived of potash. These results are consistent with the work of Hartt (*23, 25*), who found that potassium-deficient sugarcane stems contained more reducing sugar and less sucrose than normal stems, but that both sugars were low when the plants were further deprived of potash. McKaig and Hurst (*31*) reported essentially the same effects, noting that application of potassium chloride to potash-deficient plants produced an increase in sucrose and a decrease in reducing sugars in the stems of sugarcane.

At first it might seem that the greater sugar content of the juice of fruits from the "no-potash" plots indicates that lack of potash in the soil is correlated with a tendency toward increased sugar formation in the fruit; however, it must be kept in mind that previous results obtained from the same grove have shown that decreased yield and smaller size of fruit are characteristic of the no-potash plots (*6*). The mean decrease in yield from the no-potash treatment amounted to 18.5 percent. The mean increase in the total sugar concentration of fruit juice from the same treatment was 11.8 percent. Thus actually less total fruit sugar was found per tree in the no-potash plots than in the potash-treated plots, the slight excess sugar percentage found in fruit juice from the former treatment being greatly overshadowed by the diminished sugar production of the trees so treated.

The data presented herein agree in full with previous observations of Harding et al. (*22*), who found a seasonal increase in both reducing and nonreducing sugars in the principal varieties of oranges tested in Florida.

ASCORBIC ACID

Table 3 and figure 3 show the seasonal trend of and the effect of treatment on the ascorbic acid content of the fruit juices. Obviously when potash hunger is induced in an orange tree one of the results is a diminution in the ascorbic acid content of the fruit juice. Less ascorbic acid was found in juice from the no-potash plots than in that

from any of the potash-treated plots, when both seasonal means and final sample were considered. The differences were significant.

TABLE 3.—*Ascorbic acid per milliliter of Valencia orange juice on various dates during the maturation period, 1939–40*

[Means of plot replicates]

Treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Means
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
No potash	0.55	0.53	0.51	0.51	0.44	0.43	0.46	0.45	0.45	0.48
Medium sulfate of potash	.61	.60	.58	.58	.52	.51	.56	.52	.51	.55
Medium muriate of potash	.60	.59	.56	.57	.51	.49	.53	.50	.50	.54
Medium sulfate of potash-magnesia	.61	.58	.57	.56	.50	.49	.53	.50	.49	.54
High sulfate of potash	.61	.60	.58	.59	.51	.49	.56	.53	.52	.55
High muriate of potash	.60	.59	.57	.58	.51	.49	.54	.51	.48	.54
High sulfate of potash-magnesia	.61	.60	.58	.58	.51	.51	.56	.52	.49	.55
Differences required for significance:										
5-percent level									.027	.009
1-percent level									.041	.013

Roy and Bahrt (*42*) and Fudge and Fehmerling (*19*) have shown experimentally that proper fertilizer practices are accompanied by a maximum amount of ascorbic acid in oranges, but when there is a
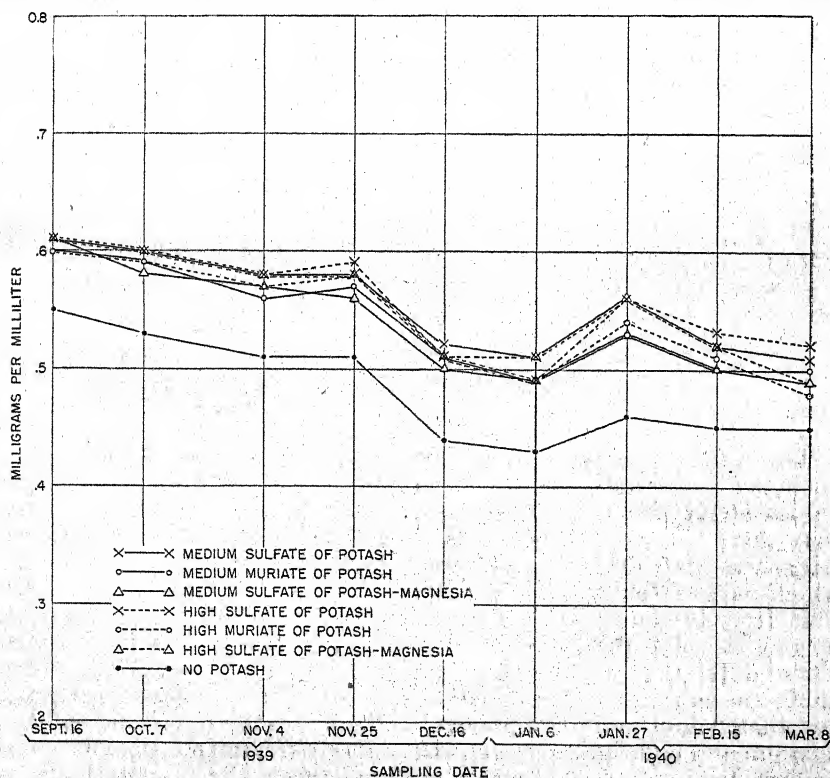
FIGURE 3.—Seasonal changes in the ascorbic acid content of Valencia oranges grown on rough lemon rootstocks, Narcoossee, Fla., 1939–40.

deficiency of any one of several elements the ascorbic acid content of the juice is lowered.

CITRIC ACID, pH, AND BUFFERS

When routine analyses of juice from mature oranges are made, the determination of citric acid consists of a titration of a sample of the juice with standardized alkali and the calculation of the acidity as citric acid. In the present experiments, however, analyses of actual citric acid were also made (table 4, fig. 4). The customary seasonal decrease in the citric acid content was apparent. Highly significantly smaller amounts of citric acid were found in the fruit juice from the no-potash plots than in that from any of the potash plots when both seasonal means and the sampling of March 8 were considered. The close agreement between the actual citric acid determined as such and the citric acid content of the juice calculated from the titratable acidity is worthy of note.

TABLE 4.—*Citric acid in Valencia orange juice on various dates during the maturation period, 1939–40*

[Means of plot replicates]

Method of determining citric acid and treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
Citric acid determined gravimetrically:										
No potash	1.76	1.42	1.32	1.19	1.09	1.00	0.95	0.85	0.84	1.16
Medium sulfate of potash	2.23	2.03	1.70	1.63	1.48	1.37	1.27	1.08	1.08	1.54
Medium muriate of potash	2.33	1.96	1.63	1.65	1.58	1.42	1.27	1.09	1.10	1.56
Medium sulfate of potash-magnesia	2.41	1.98	1.61	1.56	1.42	1.30	1.31	1.12	1.11	1.54
High sulfate of potash	2.48	2.04	1.79	1.68	1.58	1.36	1.27	1.15	1.16	1.61
High muriate of potash	2.14	2.01	1.66	1.66	1.55	1.37	1.28	1.07	1.14	1.54
High sulfate of potash-magnesia	2.56	2.13	1.89	1.72	1.54	1.42	1.38	1.16	1.19	1.67
Difference required for significance:										
5-percent level									.143	.088
1-percent level									.217	.123
Titratable acidity as citric acid:[1]										
No potash	1.78	1.50	1.22	1.16	1.06	.97	.97	.86	.82	1.15
Medium sulfate of potash	2.16	1.91	1.50	1.47	1.33	1.22	1.16	1.01	.98	1.42
Medium muriate of potash	2.31	1.89	1.49	1.43	1.39	1.26	1.15	1.00	.99	1.43
Medium sulfate of potash-magnesia	2.27	1.89	1.54	1.44	1.36	1.18	1.21	1.05	.99	1.44
High sulfate of potash	2.34	1.94	1.61	1.55	1.40	1.21	1.19	1.03	1.06	1.48
High muriate of potash	2.05	1.89	1.49	1.43	1.35	1.26	1.17	.97	1.02	1.40
High sulfate of potash-magnesia	2.39	1.95	1.65	1.55	1.43	1.35	1.21	1.10	1.06	1.52
Difference required for significance:										
5-percent level									.109	.068
1-percent level									.166	.095

[1] Data of Bahrt and Roy (6).

These data on the seasonal trends and treatment differences in citric acid were accompanied by corresponding differences in the pH values of the juices, as shown in table 5, where it may be observed that there was a regular increase in the pH values of the juice with advancing maturity. Variations for any one date between treatments which received potash in the fertilizer were slight; however, the fruit juice from the no-potash treatment had a considerably lower seasonal mean pH value than did juice from any of the potash treatments. These differences were highly significant. When the harvest sample alone was considered, the differences between the no-potash treatment and the medium sulfate of potash, the high muriate of potash, and the medium and high sulfate of potash-magnesia treatments were found to be significant at the 1-percent level. Between the no-potash treat-
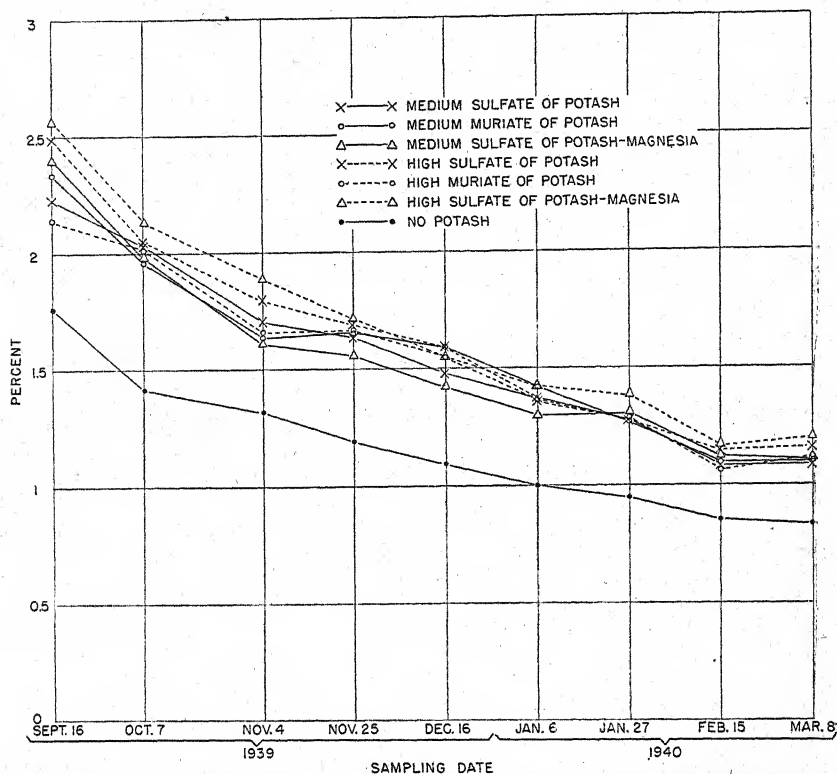
FIGURE 4.—Seasonal changes in the citric acid content of Valencia oranges grown on rough lemon rootstocks, Narcoossee, Fla., 1939–40.

ments and the high sulfate of potash treatments a significant difference at the 5-percent level was noted, but no real differences were observed in pH of juices from the no-potash treatment and the medium muriate of potash treatment.

TABLE 5.—*pH values of Valencia orange juice on various dates during the maturation period, 1939–40*

[Means of plot replicates]

Treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
No potash	2.69	2.74	2.84	2.95	3.00	3.10	3.21	3.28	3.28	3.01
Medium sulfate of potash	2.79	2.81	2.97	3.06	3.12	3 18	3.29	3.31	3.41	3.10
Medium muriate of potash	2.78	2.83	2.98	3.05	3.13	3.19	3.29	3 39	3.33	3.11
Medium sulfate of potash-magnesia	2.75	2.82	2.95	3.05	3.12	3.20	3.28	3.35	3.45	3.11
High sulfate of potash	2.79	2.83	2.96	3.05	3.13	3.24	3.30	3.31	3.36	3.11
High muriate of potash	2.81	2.81	2.99	3.08	3.12	3.20	3.33	3.41	3.37	3.12
High sulfate of potash-magnesia	2.77	2.82	2.93	3.03	3.13	3.17	3.27	3.33	3.40	3.09
Difference required for significance:										
5-percent level									.056	.029
1-percent level									.085	.041

Titration curves expressed in pH values ranging from 1.0 to 9.0 were plotted from data obtained on juice from three representative treatments at three times during the period in which samples were taken; they are presented in figure 5. It is apparent that the slopes of the titration curves (pH vs. gram equivalents of added acid or alkali) became progressively less with advancing maturity. Moreover, they were less for the juice from trees that received no potash and more for juice from trees that received a high level of sulfate of potash than for juice from plots receiving a level of sulfate of potash. These treatment differences were less pronounced as the fruit matured.

A greater slope in the pH titration curves would be expected at pH values which correspond to the pH values of citric acid. However, since the three pH values of this acid (3.08, 4.39, and 5.49) are so close together, continuous buffering throughout the entire range from pH 2.5 to 6.5 was indicated. This overlapping of the buffering regions has been pointed out by Barnes (7) in the case of malic acid in apples and tartaric acid in grapes.

The same data were replotted to show buffer indices (β) in figure 6. When these curves are studied it may be observed that the pH values at which maximum buffering occurs coincide closely with the three pK values of citric acid. As the fruit approached maturity, the peaks (β) were less pronounced, so that at maturity (March) there were only slightly distinguishable points of maximum buffering. Here, as in the pH titration curves, the influence of potash on the buffering activity is illustrated. The diminished buffer capacity associated with orange juice from no-potash plots is probably due to a combination of two contributing causes, one the lower citric acid content found in the juice from the no-potash treatments and the other the diminished cation concentration, which is also associated with such treatments.

In this connection it is desirable to point out an apparent, though not a real, disagreement with data presented by Harding et al. (22). These authors determined the buffer values of seasonal samples of orange juice and reported that the values increased with maturity, whereas the results reported herein indicate a decrease with maturity. However, they titrated only to the acid side of the original pH of the juice and to pH 1.5, which corresponds to the acidity of normal gastric juice. Their interpretation of buffer value was adopted from the definition suggested by Moore (34), which is "that amount of tenth normal HCl required to change one gram of food from its initial pH to a pH of 1.5." Since the initial pH of the juice increased with advancing maturity, the actual pH range through which the juice must pass to attain a pH of 1.5 is greater as maturity progresses, with the result that an increase occurs in the buffer value according to Moore's definition. However, when the buffer indices of orange juice are considered over the range of pH 1.0 to 9.0, it is seen that these values decrease with maturity.

The close agreement between the values obtained for citric acid by titration and for actual citric acid determined gravimetrically indicates that citric is the predominant organic acid in orange juice. This conclusion is further strengthened by the evidence that pH values at points of maximum buffering coincide closely with the pK values of citric acid. Braverman (11) reported the presence of malic, tartaric, and succinic acids in Palestine oranges in addition to citric acid, but
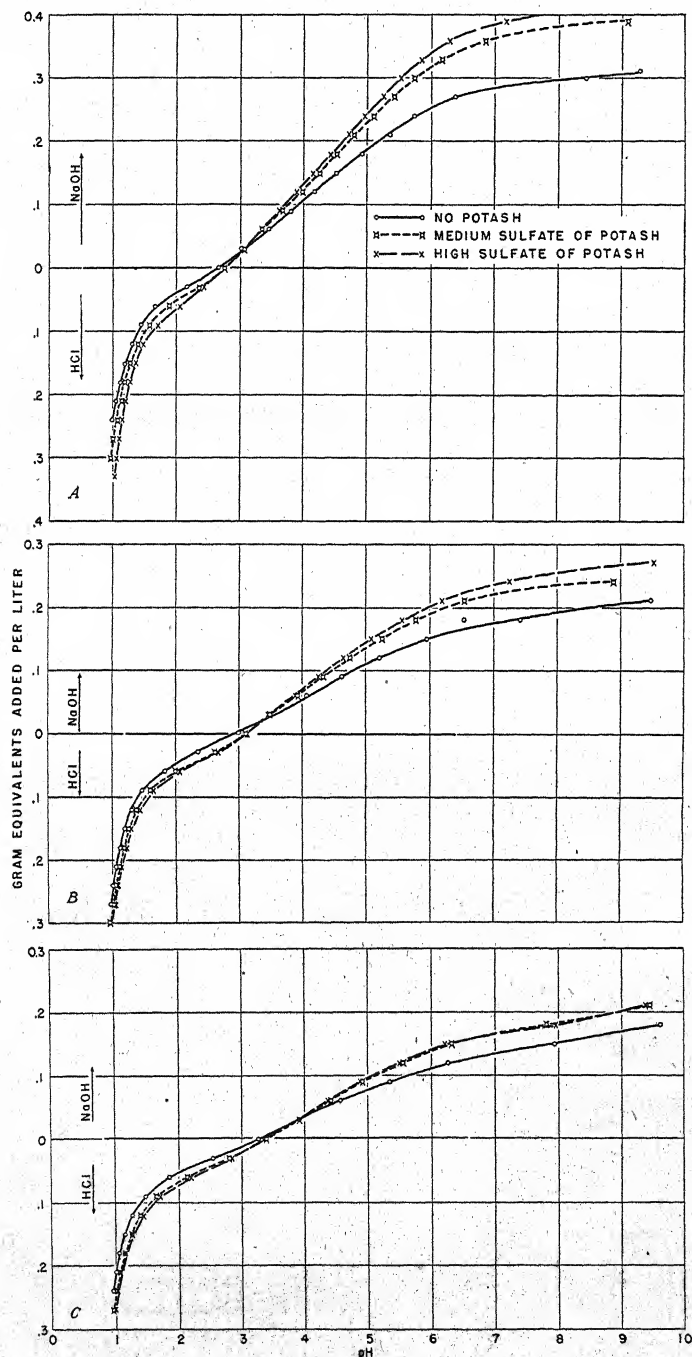
FIGURE 5.—Buffer curves for the juices of Valencia oranges from trees receiving different amounts of sulfate of potash in the fertilizer; *A*, picked September 16, 1939; *B*, picked December 16, 1939; *C*, picked March 8, 1940.
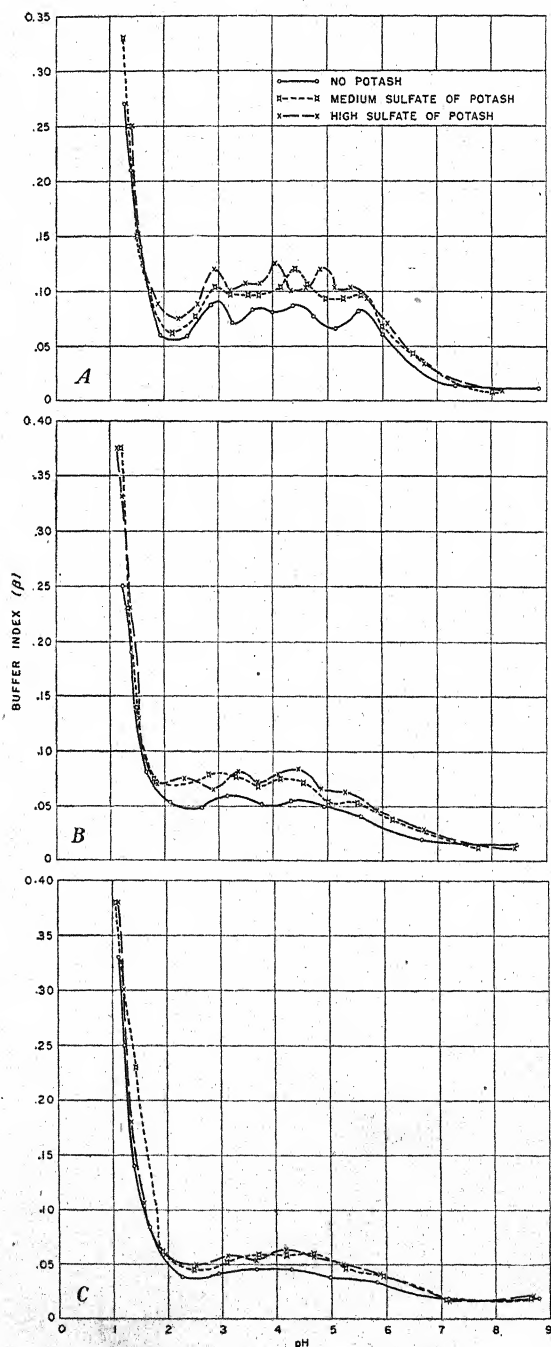
FIGURE 6.—Buffer indices of juices from Valencia oranges from trees receiving different amounts of sulfate of potash in the fertilizer: A, Picked September 16, 1939; B, picked December 16, 1939; C, picked March 8, 1940.

only in traces.   If such acids do occur in the juice of Valencia oranges, the quantity is so minute in comparison with that of citric acid that they exert no apparent influence on the buffer activity of the juice.

ASH AND ITS ALKALINITY

The ash content of the juices showed no regular seasonal trend (table 6).   However, juice from the no-potash treatment contained significantly lower (1-percent level) amounts of ash than did juice from any of the potash treatments, and during the period of sampling the amount of ash was about 30 percent lower.

TABLE 6.—*Ash in Valencia orange juice at various dates during the maturation period, 1939–40*

[Means of plot replicates]

Treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
No potash	0.221	0.174	0.183	0.160	0.187	0.203	0.241	0.214	0.229	0.201
Medium sulfate of potash	.381	.269	.289	.296	.261	.291	.331	.286	.347	.306
Medium muriate of potash	.364	.266	.283	.325	.278	.311	.388	.328	.343	.321
Medium sulfate of potash-magnesia	.349	.249	.293	.302	.256	.285	.314	.293	.337	.293
High sulfate of potash	.384	.257	.355	.339	.320	.326	.391	.319	.387	.342
High muriate of potash	.331	.265	.301	.356	.300	.288	.371	.291	.334	.315
High sulfate of potash-magnesia	.362	.264	.334	.339	.271	.323	.398	.325	.367	.331
Difference required for significance:										
5-percent level									.060	.023
1-percent level									.091	.031

The alkalinity values were significantly lower (1-percent level) in the ash from juices of fruit grown on trees receiving no potash than in that from juices of fruit grown on potash-treated trees (table 7). Alkalinity of the ash, as might be expected, varies in the same manner as the values for potassium and ash content of the various juice samples and is a function of these factors.   No correlations in ash or in alkalinity of the ash were noted with ripening of the fruit.   In general, the data reported herein on alkalinity of the ash agree with those reported on Florida oranges by Roberts and Gaddum (*41*).

TABLE 7.—*Alkalinity of the ash of Valencia orange juice at various dates during the maturation period, 1939–40*

[Means of plot replicates]

Treatment	Amount of 0.1 N hydrochloric acid required to neutralize the ash from a 100-gm. sample									
	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
	Ml.	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*
No potash	24.4	25.6	25.3	19.4	24.3	23.7	29.3	24.6	25.9	24.72
Medium sulfate of potash	43.5	39.1	41.9	38.4	44.0	41.4	44.3	38.9	42.3	41.53
Medium muriate of potash	41.8	39.3	42.4	39.9	42.9	43.6	45.6	43.1	42.0	42.28
Medium sulfate of potash-magnesia	38.5	40.9	40.8	38.0	40.5	41.3	42.0	40.0	39.6	40.17
High sulfate of potash	43.2	42.5	43.9	40.3	46.5	43.4	48.5	41.3	46.3	43.98
High muriate of potash	40.7	35.3	40.7	42.0	43.5	40.4	48.8	42.1	39.3	41.42
High sulfate of potash-magnesia	41.6	38.8	44.5	42.9	44.9	46.4	47.8	41.5	44.6	43.67
Difference required for significance:										
5-percent level									4.41	3.01
1-percent level									6.70	4.19

POTASSIUM

The amount of potassium in the juices fluctuated seasonally in a manner similar to that of the ash. Juice from the no-potash treatment contained about 60 percent of the amount of potassium found in juices derived from potash treatments. This difference is highly significant (table 8).

TABLE 8.—*Potassium content of Valencia orange juice at various dates during the maturation period, 1939–40*

[Means of plot replicates]

Treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
No potash	0.103	0.079	0.079	0.072	0.087	0.092	0.102	0.087	0.102	0.089
Medium sulfate of potash	.148	.119	.128	.132	.135	.150	.158	.137	.166	.141
Medium muriate of potash	.160	.144	.126	.137	.133	.152	.163	.152	.151	.146
Medium sulfate of potash-magnesia	.111	.125	.139	.128	.129	.141	.152	.141	.151	.135
High sulfate of potash	.175	.136	.141	.134	.147	.162	.169	.152	.165	.153
High muriate of potash	.145	.115	.131	.144	.143	.148	.159	.137	.154	.142
High sulfate of potash-magnesia	.180	.125	.138	.136	.132	.161	.168	.137	.165	.149
Difference required for significance:										
5-percent level									.0171	.0106
1-percent level									.0260	.0148

From the data presented in tables 6 and 8, it can be readily observed that potassium constitutes a greater portion of the ash of orange juice than do any of the other metals. The salts of citric acid present in the unaltered juice are oxidized to the corresponding carbonates, at the low temperatures (400° to 500° C.) employed in ashing the samples. Thus, it is evident that potassium carbonate constitutes from 70 to 80 percent of the weight of the ash.

The data in tables 6 and 8 have been employed in calculating the percentages of potassium in the ash. These percentages, presented in table 9, show no significant differences between the seasonal-mean potassium content of the ash from juice from the no-potash trees and that from the potash-fed ones. There was, however, a large percentage difference with respect to both the ash and potassium between juices from the no-potash and from the potash-fed trees. Under the conditions imposed in this study with regard to soil and soil amendments, it is apparent that the potassium content of the juice is proportional to the content of the total of the other inorganic constituents of the juice. This finding is entirely in keeping with data reported by other workers (*38, 41*), who found the potassium content of the ash of orange juice to be 42 to 45 percent, but it is in direct contrast to the data from the inorganic analyses of the leaves taken from the same trees. In the latter case a distinct antagonism was apparent between the amounts of potassium and calcium in the leaf. In leaves in which the potassium content was low the calcium was found to be much higher than normal. The analyses of leaf and juice indicate that the fluctuation in mineral composition, as affected by available soil nutrients, is reflected to a great extent in the leaf, which acts as a regulating mechanism or buffer and serves to maintain a fair degree of constancy in the inorganic constitution of the juice.

TABLE 9.—*Potassium content of the ash of Valencia orange juice at various dates during the maturation period, 1939-40*

[Means of plot replicates]

Treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
No potash	46.6	45.4	43.2	45.0	46.5	45.3	42.3	40.7	44.5	44.4
Medium sulfate of potash	38.9	44.2	44.3	44.6	57.5	51.5	47.7	47.9	47.8	47.2
Medium muriate of potash	44.0	54.1	44.5	42.2	47.8	48.9	42.0	46.3	44.0	46.0
Medium sulfate of potash-magnesia	31.8	50.2	47.4	42.4	50.4	49.5	48.4	48.1	44.8	45.9
High sulfate of potash	45.6	52.9	39.7	39.5	45.9	49.7	43.2	47.6	42.6	45.2
High muriate of potash	43.8	43.4	43.5	40.5	47.7	51.4	42.9	47.1	46.1	45.2
High sulfate of potash-magnesia	49.7	47.3	41.3	40.1	48.7	49.8	42.2	42.2	45.0	45.1
Difference required for significance:										
5-percent level										3.51
1-percent level										4.89

RATIO OF REDUCING TO NONREDUCING SUGARS

The ratios of reducing to nonreducing sugars (table 10; fig. 7), showed a uniform decrease with advancing maturity until January,
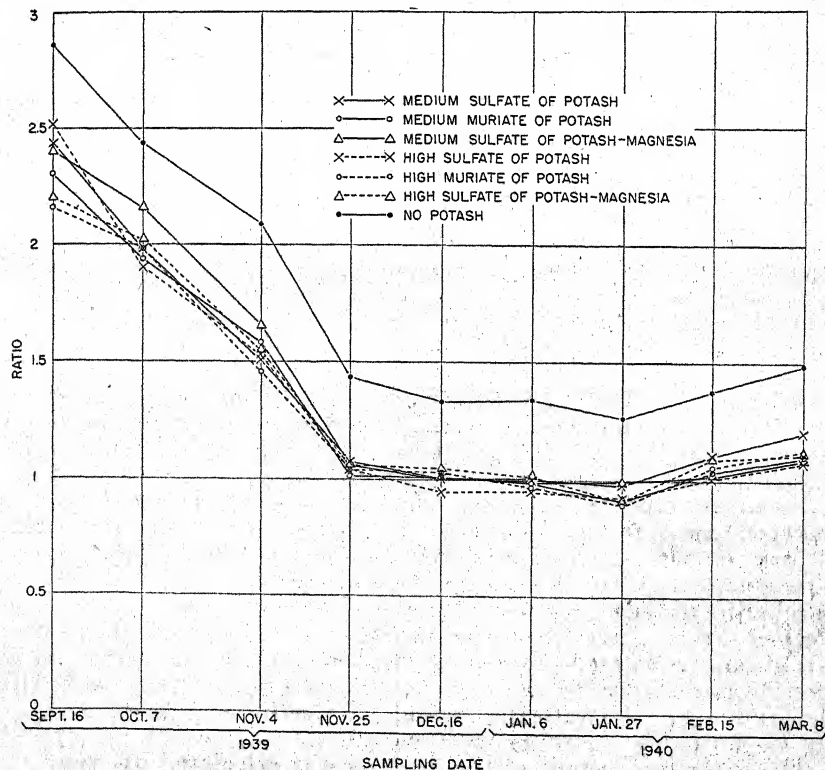
FIGURE 7.—Seasonal changes in the ratios of reducing to nonreducing sugars in the juices from Valencia oranges grown on plots with different amounts of potash and with potash derived from different sources, 1939-40.

when a minimum was reached; after that the ratios increased slightly. The juice from fruit from the no-potash plots contained a significantly

higher ratio of reducing to nonreducing sugars than the juice from fruit from any of the other treatments on each sampling date, although the trends were in the same direction. As the ratios decreased with maturity of the fruit, the pH values of the juice increased.

TABLE 10.—*Ratio of reducing to nonreducing sugars in Valencia orange juice at various dates during the maturation period, 1939–40*

[Means of plot replicates]

Treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
No potash	2.86	2.46	2.08	1.43	1.33	1.34	1.26	1.38	1.49	1.74
Medium sulfate of potash	2.43	1.98	1.51	1.07	1.01	1.00	.98	1.11	1.20	1.37
Medium muriate of potash	2.30	1.94	1.58	1.01	1.01	.98	.91	1.03	1.10	1.32
Medium sulfate of potash-magnesia	2.41	2.16	1.65	1.06	1.01	.99	.99	1.02	1.09	1.38
High sulfate of potash	2.52	1.90	1.53	1.04	.95	.95	.92	1.01	1.08	1.32
High muriate of potash	2.16	1.98	1.46	1.04	1.03	.97	.90	1.06	1.12	1.30
High sulfate of potash-magnesia	2.20	2.02	1.54	1.06	1.04	1.01	.91	1.09	1.11	1.33
Difference required for significance:										
5-percent level									.186	.083
1-percent level									.283	.116

A scatter diagram in which ratios of reducing to nonreducing sugars were plotted against the pH values of the juices was made (fig. 8, *A*). Since it resembles an exponential type of curve and pH is a logarithmic function, the data were replotted, graphing the logarithms of the ratios of reducing to nonreducing sugars against pH. The regression line was calculated and inserted as shown in figure 8, *B*. The equation for the line was found to be

$$E = 1.8778 - 0.5692X$$

where

$$E = \log \frac{\text{reducing sugars}}{\text{nonreducing sugars}}$$

$$X = \text{pH.}$$

A highly significant negative correlation coefficient (r) of -0.845 was found, showing a high degree of correlation between the pH of the juice and the logarithm of the ratio of reducing to nonreducing sugars.

In an attempt to determine whether there was a causal relation between these factors, synthetic solutions were made, containing 10 percent sucrose, citric acid varying from 1.0 to 2.6 percent, and potassium carbonate, buffered in steps of 0.2 unit from pH 2.0 to 3.4. Saccharimeter readings were taken periodically over a period of 3 weeks. From these data the rate of inversion was determined. No attempt was made to control the temperature, since the experiment was designed to simulate actual conditions which exist with the fruit. The laboratory temperatures fluctuated between 20° and 30° C. during the course of the experiment.

The velocity constant of the reaction was calculated by using the formula for a second-order reaction according to Rice (*40, p. 123*):

$$k = \frac{1}{at} \, ln \, \frac{b}{b-x} \, ^{7}$$

[7] Scatchard (*46, 47*) states that the characteristics of the reaction more nearly approximate those of a sixth-order reaction.
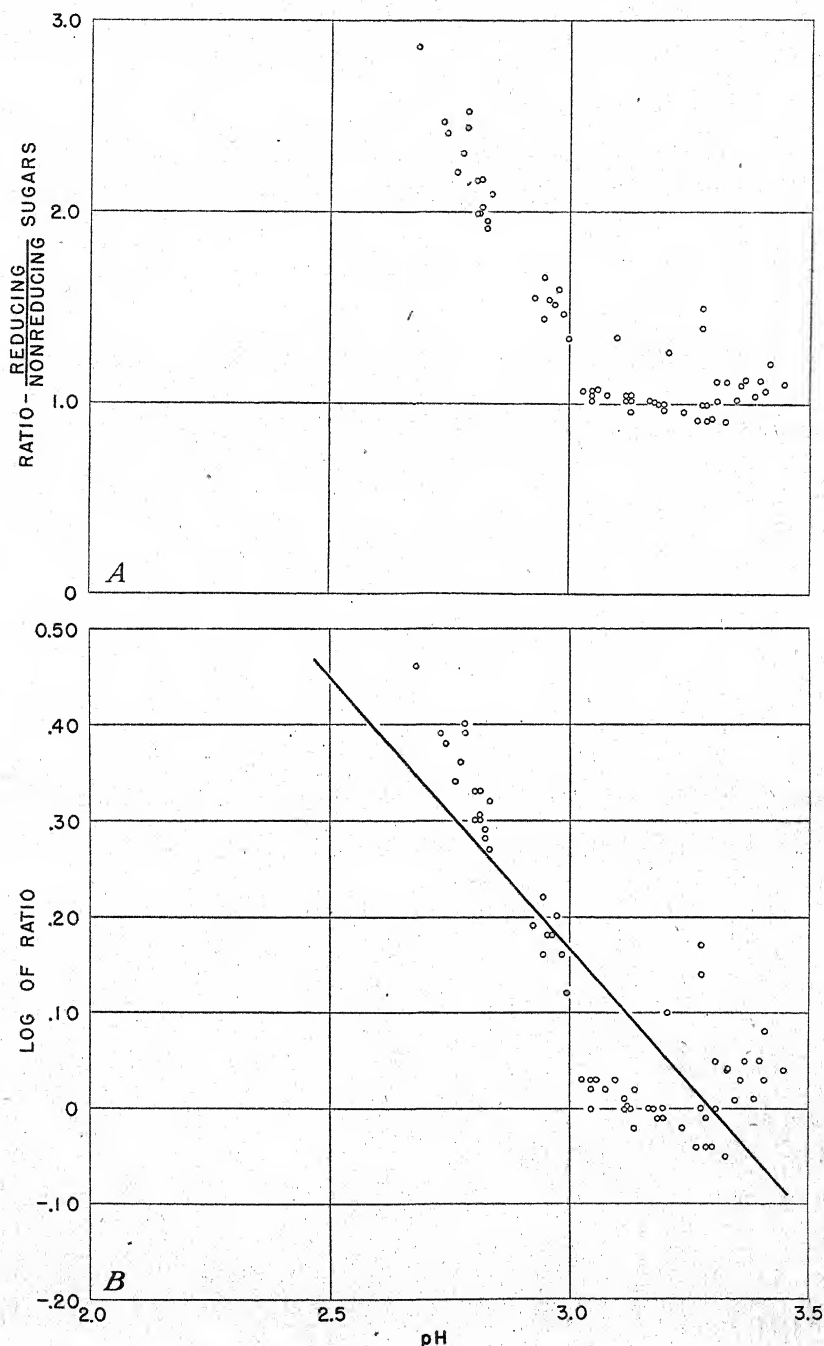
FIGURE 8.—Relation between the hydrogen-ion concentration (pH) of Valencia orange juice and the ratio of reducing to nonreducing sugars, 1939–40: *A*, Scatter diagram; *B*, regression line of pH vs. logarithm of ratio.

where

k = the velocity constant
a = the hydrogen-ion concentration
t = the time in minutes
b = the initial concentration (molar) of sucrose
x = the number of mols of sucrose inverted in time, t.

The mean value for k was found to be 0.0094 under the conditions imposed.

Thus, at a pH of 2.0 it was found that 50-percent inversion of sucrose (i. e., a ratio of reducing to nonreducing sugars of 1.0) was accomplished in 5.1 days, at pH 3.0 in 51 days, and at pH 4.0 in 510 days.

Further calculations showed that at a pH of 2.69, the lowest mean pH value obtained on any juice during the study, which represents the pH value of juice from the first sampling of fruit from the no-potash treatment, the mean ratio of reducing to nonreducing sugars was 2.84. If the pH and the initial sucrose concentration of this juice had remained constant, such a ratio would have been attained in 50 days. Similarly, for a pH of 2.81 and a ratio of 2.08, which represent the highest pH and the lowest ratio, respectively, for the initial sampling of juice from oranges picked from the high muriate of potash treatment, 54 days would have been required for attainment of that ratio under the same conditions.

It should be kept in mind, however, that the conditions that exist in vivo are subject to several fluctuations. Thus, the initial sucrose concentrations are constantly changing with advancing maturity, the acidity of the juices is slowly decreasing during the same period, and the temperature fluctuations are considerable. Since each of these factors is of major importance in the ripening process and in influencing the amount and extent of inversion, it is practically impossible to formulate the conditions that exist within the fruit. However, since the degree of inversion bears such a close relation to the pH value of the juices, the ratios, and the length of time required for such inversion, and, further, since the actual ratios found are easily within the range calculable at the pH values that exist, it is postulated that the sugar which enters the fruit by way of the vascular tissue (*32*) is composed entirely of sucrose. Such sucrose in fruit is partially inverted to the extent found by the catalytic action of the hydrogen ions derived from the dissociation of the citric acid.

While the latter statement does not preclude the possibility of inversion due to enzyme action, it is believed that such inversion can occur without the presence of hydrolytic enzymes; this is in keeping with the conclusions of Andre (*3*).

Thus, if this postulate is tenable, relatively rapid inversion of sucrose occurs in very immature fruit owing to the high acidity of the juice of such fruit, and there is consequently a high ratio of reducing to nonreducing sugars. As maturity progresses, more sucrose is translocated to the fruit and is hydrolyzed at progressively slower rates as the citric acid diminishes and the pH values increase. These changes make for a lower ratio because the invert sugar portion (the numerator) is increasing at a slower rate than the unhydrolyzed portion (the

denominator).   Another factor which undoubtedly influences this seasonal trend toward lower ratios is that during the period of maturation (December and January), when the ratios attain their lowest values (table 10), the prevailing temperatures are at a minimum. Since the $Q^{10}\left(\dfrac{k35}{k25}\right)$ of acid hydrolysis of sucrose is stated to have a value of 4.1 (*40*), the temperature effect in retarding or slowing hydrolysis is considerable.   In February and March the slight upward trend in the ratios can best be explained by the effect of the higher temperatures which prevail in the State at this time of year.

DISCUSSION

Since the legal standard of maturity for oranges is based upon the ratio of total soluble solids (consisting for the most part of sugars) to acid content of the juice, and since it has been shown that an induced potash deficiency in the tree is accompanied by a higher concentration of sugar in the fruit juice, together with a decrease in the citric acid content, it is apparent that legal maturity is hastened by withholding potash from the trees.   In the case of total deprivation of potash, however, the detrimental effects produced more than offset any advantages to be gained by causing fruit to attain a legal total-soluble-solids-acid ratio sooner than normal.   These relations have been discussed in an earlier publication (*6*).

Again, early maturing of Valencia oranges in Florida is not particularly desirable from an economic standpoint.   The sale of such early fruit would necessarily enter into competition with midseason fruit. The practical aspect of these findings probably lies in their application to early fruit, exemplified in the State by the Parson Brown variety. It is highly probable that, by judicious application of the potash ingredient of the fertilizer, legal maturity could be controlled to a large extent.   Indeed, it has been shown experimentally (*6*) that when all the potash was applied to Parson Brown orange trees in the fall application rather than in the customary three applications, legal maturity (solids-acid ratio of 8 to 1) was attained 2 to 3 weeks earlier than in fruit which had been fertilized in the customary manner.   It is significant, also, that when such practice was adopted no measurable decrease in size or in yield of fruit was observed.

No differences of consequence were noted with regard to the source of potash in any of the treatments.   The economic significance of this observation is important.   The greater part of the potash produced in the United States consists of the muriate salts.   The cost of potash from this source is considerably less than that of potash obtained from foreign deposits as the sulfate, or domestic sulfate of potash produced by acid-conversion and base-exchange processes.   In addition, foreign potash is difficult to procure at the present time.   The results obtained in this experiment indicate that for fertilization of Valencia oranges, the muriate salts constitute as desirable a source of potash as the sulfate or the sulfate of potash-magnesia.   In 1937 Florida alone consumed 35,813 tons of potash as fertilizer (*15, p. 401*) and a considerable part of this was applied to citrus groves.   Thus, the use of the less expensive potash from domestic sources is a matter of practical importance.

Another point brought out by this experiment is the fact that the critical level of potash fertilization for citrus lies at or below the medium level of application described in these studies; i. e., an annual application of 1.7 to 2.3 pounds of potash. This deduction is readily apparent from the observation that yield and quality of the fruit, as well as some of the biochemical characteristics of the juice, were not materially improved by the application of a fertilizer of higher potassium content. The few benefits obtained were hardly of sufficient value to warrant the additional cost of application of more than the amount of potash contained in the medium mix.

The influence of the potassium content on the hydrogen-ion concentration of the juice is very marked. Ordinarily, if the pH values of two orange juices are compared, the juice that contains the larger quantity of citric acid will also be found to contain the greater concentration of hydrogen ions.

An examination of the data presented herein pertaining to the citric acid content and the pH values of the various juices shows that this general relation does not hold, but that the pH value of the juice is a function of two factors, depending on the citric acid concentration and the ash. It is apparent that on any sampling date juice from the no-potash plots contained considerably less potassium and less citric acid and yet its mean pH value was lower by about 0.10 pH unit than that of juice from any of the potash treatments. This difference becomes more apparent when it is remembered that a difference of 0.10 pH unit means approximately a 20-percent difference in hydrogen-ion concentration.

The increase in the pH value of juice which contains an adequate amount of potassium over that which is deficient is directly attributable to the potassium content of the juice; the differences undoubtedly would be more accentuated were the citric acid content of the juices equal. Thus, juice which contains an adequate amount of potassium can be expected to have a higher pH value than potassium-deficient juice of the same citric acid content.

SUMMARY

Biochemical studies were made of the juice from Valencia oranges taken from trees that received no potash and from trees that received applications of 1.7 to 2.3 and from 2.7 to 3.6 pounds annually of potash per tree from three sources, namely, muriate of potash, sulfate of potash, and sulfate of potash-magnesia.

Potassium deficiency induced by withholding potash for 5 to 5½ years was accompanied by the following changes in the composition of the fruit juice, when compared with fruit juice from trees supplied with potash in the fertilizer: (1) An increased concentration of reducing sugars; (2) an increased concentration of total sugars; (3) a decreased concentration of ascorbic acid; (4) a decreased concentration of citric acid; (5) a decrease in the buffer index of the juice; (6) a decrease in the pH value of the juice; (7) a decrease in the percentages of ash and potassium and in the alkalinity of the ash; (8) an increase in the ratio of reducing to nonreducing sugars.

Inasmuch as legal maturity is based on the ratio of total soluble solids to acid in the juice, potassium deficiency induces a 2- to 3- week earlier onset of legal maturity.

The belief commonly held that muriate of potash in the fertilizer exerts a harmful effect on the quality of Valencia oranges is not substantiated by the results of these experiments. No differences attributable to the source of potash were found in the juice of oranges from plots that received muriate of potash, sulfate of potash, or sulfate of potash-magnesia.

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INHERITANCE OF ASCORBIC ACID CONTENT IN SNAP BEANS [1]

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INTRODUCTION

In a program of breeding hardy, productive snap beans (*Phaseolus vulgaris* L.) adapted for growing in the southeastern United States, it was found that unless some attention were given to ascorbic acid (vitamin C) content the hardy strains developed were likely to have less ascorbic acid than varieties commonly grown in the Southeast, such as Stringless Black Valentine and Bountiful.[2] The decrease in ascorbic acid content was probably due to the fact that the hardy parents, U. S. No. 5 Refugee and closely related lines, have an average ascorbic acid content lower than that of the commonly grown varieties. Some crosses appeared to be segregating widely, so a genetic study was undertaken in the hope that a basis might be found for solving the breeding difficulty. The investigation was made in 1942 at the United States Regional Vegetable Breeding Laboratory, Charleston, S. C.

MATERIALS AND METHODS

Notes were kept on broken and diseased plants, but data obtained from these plants were ordinarily not used in compiling the results. The inclusion of data from broken and diseased plants increases the variability, since when injury first occurs the ascorbic acid content is usually high but with the death or senescence of the plant ascorbic acid may be very low. Where a small amount of some disease was noted it was indicated as being present, but the data were not discarded. Little breaking or disease was observed.

The cross used in this study was U. S. No. 5 Refugee × Blue Lake and its reciprocal. Preliminary studies of F_2's of this cross were made in the spring of 1942. In the summer 4 F_3 families derived from F_2 plants having very high or very low ascorbic acid content were grown. In the fall a reserve lot of F_2 material, 25 F_3 families, and the same number of the reciprocal were studied. Two substrains of each of the parent varieties, as well as 2 F_4 families of the cross and 1 of the

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reciprocal, were also studied in the fall. Single-plant selections of the snap varieties were used in the cross and the same ones as controls when the F_2 and F_3 progenies were being observed.

Approximately 75 seeds were planted in each 32-foot row, and samples were taken as soon as the pods were considered to have arrived at an edible stage. Pods of U. S. No. 5 Refugee and Blue Lake were harvested as checks each day that pods of the hybrids were harvested. Harvesting usually began at 7:30 a. m. and was complete by 11 a. m. Ascorbic acid determinations were made immediately or else on material held not longer than 24 hours with high humidity at a temperature of 36° F. Records of all plants were kept on an individual basis, and an effort was made to obtain samples from each plant at two different periods, preferably about 1 week apart. A study of the influence of size and position of pods on the plant on the ascorbic acid content of the U. S. No. 5 Refugee parent was also made.

Approximately 2,300 ascorbic acid determinations were made in this work in 1942, of which about 500 made on the spring crop were considered as only exploratory in nature.

A modification of the Morell [3] method was used for the determination of ascorbic acid. From 25 to 40 gm. of the bean pods was extracted with 200 ml. of 1-percent metaphosphoric acid for 2 minutes in a Waring blender. The extract was filtered through a fluted Whatman No. 12 filter paper, and the first portion of the filtrate was discarded. A 1-ml. aliquot was placed in a colorimeter tube containing 10 ml. of a solution of 2,6-dichlorophenolindophenol (concentration, 17 mg. per liter) and read in an Evelyn photoelectric colorimeter within 30 seconds. Satisfactory readings were obtained when the concentration of the extract was between 20 and 70 micrograms of ascorbic acid per milliliter. Moisture determinations were made, and the dilution effect of the moisture in the sample was considered in calculating the ascorbic acid.

The procedure just described eliminates the use of buffers without loss of accuracy and increases the number of determinations that can be made per day.

RESULTS

In the spring of 1942, the F_2 progenies from U. S. No. 5 × Blue Lake gave a mean ascorbic acid content of 23.84 mg. per 100 gm. (fresh weight), with a standard deviation of 5.54, and those from the reciprocal cross gave 22.45 ± 5.69 mg. The ranges were 11.2 to 28.5 and 10.9 to 33.4 mg., respectively. Owing to an accident, the strains used as parents did not mature at the same time as the F_2 so that other U. S. No. 5 Refugee and Blue Lake strains were used as substitutes for the parents. These gave 22.01 ± 3.42 and 18.94 ± 2.47 mg., respectively.

In the summer of 1942, as previously stated, a small planting of parents and F_3 seed from the high and low lines of the spring-grown F_2 of the U. S. No. 5 Refugee × Blue Lake and reciprocal was made. The U. S. No. 5 Refugee parent averaged 19.5 mg. per 100 gm. in ascorbic acid and the Blue Lake parent 15.1 mg. per 100 gm. The averages for the high and low lines of the cross and its reciprocal were 11.0

[3] MORELL, S. A. RAPID PHOTOMETRIC DETERMINATION OF ASCORBIC ACID IN PLANT MATERIALS. Indus. and Engin. Chem., Analyt. Ed. 13: 793–794, illus. 1941.

and 31.7 mg. and 11.8 and 32.8 mg., corresponding to spring values of 11.2 and 28.5 mg. and 10.9 and 33.4. mg., respectively. The requirement for significant difference at the 5-percent level was 2.2 for parents and hybrids when combined in a variance analysis.

In the fall of 1942 the mean ascorbic acid value for the F_2 of U. S. No. 5 Refugee × Blue Lake was 19.5 mg. per 100 gm. with a standard deviation of 4.30, the range of values being from 12.2 to 32.4 mg.; for the reciprocal the mean value was 16.0 ± 3.08 mg., with a range of 10.5 to 22.6 mg. These values are fairly close together, so the data were combined for presention in figure 1, *C.*
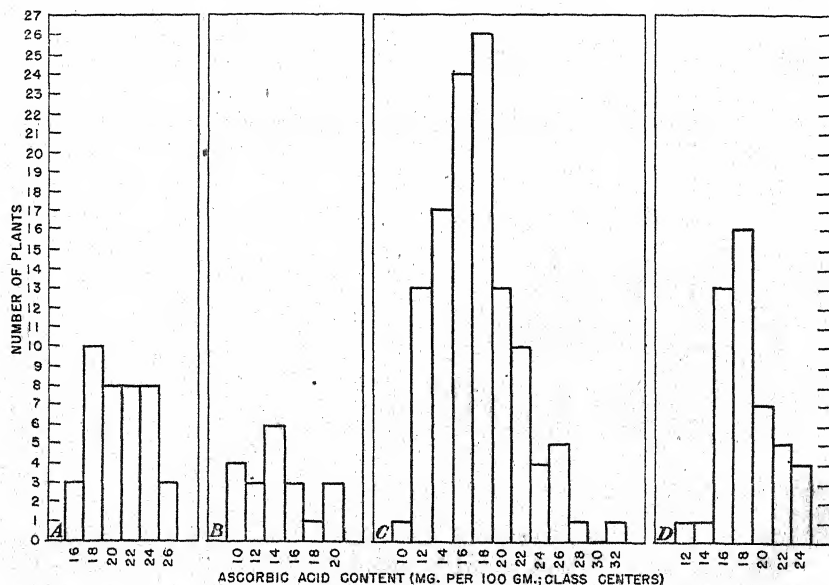
FIGURE 1.—Distribution with respect to ascorbic acid content of (*A*) U. S. No 5 Refugee plants, (*B*) Blue Lake plants, (*C*) F_2 plants of U. S. No. 5 Refugee × Blue Lake and the reciprocal, and (*D*) F_3 families of the same crosses. Mean ascorbic acid content: *A*, 20.9 ± 2.97; *B*, 14.2 ± 3.20; *C*, 17.7 ± 4.03; *D*, 17.5 ± 4.39.

The F_3 of the cross U. S. No. 5 Refugee × Blue Lake had a mean ascorbic acid content of 17.9 mg. per 100 gm., with a standard deviation of 4.25, and the F_3 of the reciprocal cross had a value of 17.1 ± 4.49 mg. The range was from 7.6 to 31.6 mg. for the cross, and from 6.0 to 35.0 mg. for the reciprocal based on single plants. The range of F_3 family means was 13.3 to 23.7 for the cross and 13.7 to 22.8 for the reciprocal. Since the values for this cross and its reciprocal are very close, they were combined to give the results shown in figure 1, *D.*

One F_4 family from the cross showed a mean of 12.0 mg. per 100 gm. and one from the reciprocal showed 11.8, while another family from the cross averaged 30.0 mg.

Two substrains of the U. S. No. 5 Refugee gave ranges of 16.7 to 26.8 mg. per 100 gm. and 16.4 to 25.2 mg., with means of 20.9 and 20.9 mg. and standard deviations of 2.77 and 2.89, respectively. Two substrains of Blue Lake gave ranges of 9.2 to 16.7 mg. and 11.9 and 19.8

mg., with means of 11.8 and 15.3 mg. and standard deviations of 2.73 and 3.04, respectively. For each variety the two substrains were combined in a single distribution in figure 1, *A* and *B*.

At one time during the harvesting season a study was made to determine whether the size of pods and their position on the plant influenced the ascorbic acid content. This study was confined to the U. S. No. 5 Refugee parent. Single plants were divided into an upper and lower part and the pods from each part were divided into medium and large. The medium pods averaged 17.5 mg. of ascorbic acid per 100 gm., and the large pods 20.5 mg.; the upper pods averaged 18.7 mg. and the lower pods 19.4 mg. The requirement for significant difference is 1.3. The average for medium pods was the same whether from the upper or the lower part, but large pods from the lower part averaged 21.2 mg. as compared with 19.8 for large pods from the upper part, with 1.9 required for significant difference.

DISCUSSION

The means for ascorbic acid content of the F_2 and the F_3 populations of the fall crop were very close to the mean of the U. S. No. 5 Refugee and Blue Lake parents. The variance (standard deviation squared) for the parents was approximately half the variance for the segregating generations (9.38 vs. 16.21 for F_2 and 19.25 for F_3). From these values it might have been possible to calculate the number of genes involved, except that there is evidence of transgressive segregation. Both F_2 and F_4 gave ascorbic acid values beyond the upper range of the parents. Reynard and Kanapaux,[4] in a preliminary genetic study of ascorbic acid content of tomatoes, found the content of hybrids intermediate between those of the two parents.

Because of unfavorable weather conditions, snap beans are ordinarily not grown in the Charleston, S. C., area during the summer months, but owing to the possible breeding value of some of the lines high in ascorbic acid content, a small summer planting was made of parents and of high and low F_3 lines of the cross U. S. No. 5 Refugee × Blue Lake and of the reciprocal cross. These F_3 lines gave means near the values obtained for the corresponding F_2 in the spring. Reduction in variance is probably due to uniformity of harvest (all samples in 1 day) and to an approach to genetic uniformity for high and low ascorbic acid values in the F_3 lines.

The study of influence of position and size of pods on ascorbic acid content gave an error variance of 5.02, indicating that the remainder of the U. S. No. 5 Refuge variance for the fall of 1942 (7.84−5.02=2.82) was due to the influence of time of picking and other factors within the picking season. Since these other factors cannot be readily controlled it is necessary to increase the number of observations.

[4] REYNARD, G. B., and KANAPAUX, M. S. ASCORBIC ACID (VITAMIN C) CONTENT OF SOME TOMATO VARIETIES AND SPECIES. Amer. Soc. Hort. Sci. Proc. 41: 298–300. 1942.

SUMMARY AND CONCLUSIONS

In a genetic study of snap beans involving considerable preliminary work it was found that in the cross U. S. No. 5 Refugee $\times$ Blue Lake and its reciprocal the quantity of ascorbic acid is a heritable character and that there is evidence of transgressive segregation. Although the spring-grown F_2 is considered as giving only preliminary information it indicated transgression, and this indication was confirmed by a summer-grown crop of F_3 lines selected for high and low ascorbic acid content. These high and low F_3 lines were all significantly different at the 1-percent level from the parent lines and close to the values observed for the corresponding F_2 plants.

In the fall the F_2 population again indicated transgressive segregation, but the F_3 means did not. The 50 F_3 families taken strictly at random from the F_2 plants did not include any families with average values significantly above or below the parental values. However, some F_3 families had as wide a range as the F_2. Some additional evidence of transgression was found in 3 F_4 families.

It was found that much of the variability in ascorbic acid content of pods of U. S. No. 5 Refugee was due to size and position on the plant, i. e., a variance of 5.02 for size and position with a remaining variance of 2.82 for all other factors involved during the fall of 1942.

TOTAL AND FREE AMYLASE CONTENT OF DORMANT CEREALS AND RELATED SEEDS[1]

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INTRODUCTION

Studies of the diastatic power of dormant cereal seeds have been made by a number of investigators, mostly within recent times. These studies have been concerned largely with barley because of the possible correlation between the amylase content of the dormant seeds and that of the malt.

Most of the past studies have dealt with three problems: (1) The role of variety in determining the variation in the amylase content of barleys; (2) the mechanism of stimulation of the diastatic activity by natural substances developed in the seeds during germination or by extraneous substances added to the dormant grains or malts during the extraction period; (3) the nature of the amylase of dormant seeds and malts.

Myrbäck (*29*)[2] after extensive study came to the conclusion that when wide allowances are made for environmental factors such as climate, rainfall, soil, and methods of cultivation, variety is definitely one of the factors responsible for the variation in the amylase content of barley. Chrzaszcz and Sawicki (*14*), on the other hand, claim that extensive studies carried out by them showed no variation in the amylase content of barley that could be attributed to the influence of variety. Myrbäck's views seem to be supported by Mangels (*28*), Dickson and associates (*16*), Hills and Bailey (*19*), and Sallans and Anderson (*33*).

The mechanism of activation of the diastatic power in cereals and malts, studied first by Ford and Guthrie (*17*), has been the subject of wide discussion. Two recent views on this subject are expressed by Myrbäck and Örtenblad (*30*) and by Chrzaszcz and Janicki (*7, 8*). According to Myrbäck and Örtenblad the amylase in cereals is combined with proteins and the activator, which is a proteinase, splits up the proteins and sets the amylase free. According to Chrzaszcz and Janicki there are present in the seeds inhibitors (sisto-substances) which interfere with the functioning of the amylase. The activators (the eleuto-substances) counteract the effect of these natural inhibitors. Added extraneous activators act as eleuto-substances.

The nature of the amylase found in dormant seed has also been a subject of wide study. It is universally recognized that the amylase in dormant seeds is mostly beta-amylase. There is, however, a disagreement among investigators as to whether or not the alpha-amylase, which is found in appreciable quantities in barley malt, is

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[1] Received for publication, May 21, 1943.
[2] Italic numbers in parentheses refer to Literature Cited p. 199

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not also found in small quantities in dormant seeds. Nordh and Ohlsson (31), Lüers and Rümmler (27), and Chrzaszcz and Janicki (9) believe that alpha-amylase is entirely absent from dormant seeds. On the other hand, Józsa and Gore (24), Sandstedt and associates (34), and Shellenberger and Bailey (35) claim that it is present in dormant seeds. Hills and Bailey (19) recently recorded quantitative measurements of small amounts of alpha-amylase in several varieties of barley.

In the present investigation the amylase content of dormant seeds was studied as a property of cereals in general, and not with regard to their malting properties only. The amylase content of cereals used for bread making, such as wheat and rye, may prove to be one of the factors that determine the baking qualities of their flours. The amylase content of other seeds may be a factor in determining their suitability as flour substitutes and in developing the baking procedure to be adopted. A more comprehensive knowledge of the amylase content of seeds may also throw light on the dormancy of winter grains and the problem of vernalization.

MATERIALS AND METHODS

The principal cereals studied included four varieties each of barley, wheat, rye, and oats, two varieties of corn, and one variety of rice. By way of comparison one variety of buckwheat and two varieties each of soybeans, sorghums, and cowpeas were also studied. All seeds were of known origin and were obtained from the Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture. The ordinary white flour used was obtained on the open market.

The extraction of the free amylase was carried out according to the approximate procedure of the British Institute of Brewing (20). Two grams [3] of the ground dormant seeds were weighed out into 125-ml. Erlenmeyer flasks. Sixty milliliters of distilled water were added to each flask and the contents stirred with a glass rod. Ten drops of toluene were added to each flask. The flasks were then stoppered and allowed to stand in a constant-temperature room at 70° F. and shaken every half hour. The extraction of the total amylase was carried out in the same way as the free amylase except that 60 ml. of a half-percent solution of papain were used instead of distilled water. The extractions of the amylase from barley and rye, shown in tables 1 and 3, were carried out in duplicate. The results obtained from the duplicate extractions agreed reasonably well. The results recorded in the other tables were obtained from single extractions. Frequent repetitions, however, were made to check the results.

For the determination of the diastatic power [4] of the extracts 3 ml. of the supernatant clear liquid were withdrawn with a pipette from each Erlenmeyer flask and placed in a corresponding 200-ml. volumetric flask containing 100 ml. of a 2-percent buffered solution of soluble starch. This was repeated at various intervals such as 3, 24, 48, and 72 hours. The contents of the volumetric flask were well shaken and

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[3] For malt extractions 1-gm. charges were used.
[4] The terms "diastatic power," "amylase," "diastase," and "saccharifying power" are used interchangeably. The terms "free" and "total" designating respectively the amylase extracted with distilled water and with the aid of papain, were first used by Myrbäck (29).

allowed to stand for 1 hour at 70° F. in a constant-temperature room. Six milliliters of a half normal solution of sodium hydroxide were then added to each volumetric flask in order to stop the saccharifying action. The volumetric flasks were then allowed to regain room temperature and made up to volume.

The maltose determinations were made by the Lane and Eynon method (*25*). With material which had a low amylase content, the excess of Fehling's solution after the addition of 25 ml. of the hydrolyzed starch solution was titrated back with a standardized half-percent solution of dextrose. The results were converted into degrees Lintner and represent averages of duplicate determinations.

DIASTATIC POWER OF DORMANT SEEDS

The ground materials were extracted as just described above and the total and free amylase strength for every extraction period was determined, the free amylase being also computed in percentages of total diastatic power. The results are given in tables 1 to 6.

Barley, wheat, and rye possess an appreciable diastatic power (tables 1, 2, and 3). The extraction of free amylase was not complete after 24 hours, the usual time limit used by numerous investigators. In these experiments maximum extraction was not reached until about 3 days. This is true also for total amylase except for wheat, in which the maximum extraction was reached in 3 hours or less. Rye and wheat seem to contain more total and free amylase than barley. Rye has a higher free diastatic power than wheat, but the total diastatic strength of wheat is higher than that of rye.

TABLE 1.—*Development of total and free diastatic power in degrees Lintner in 4 varieties of dormant barley during different periods of extraction*

Variety	Diastatic power for extraction period of—											
	3 hours			26 hours			74 hours			98 hours		
	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total
	°L.	°L.	Percent	°L.	°L.	Percent	°L.	°L.	Percent	°L.	°L.	Percent
Wisconsin Winter	77.2	43.0	55.7	84.0	49.8	59.3	90.5	60.6	67.0	88.5	60.3	68.1
Tennessee Winter	71.1	40.1	56.4	75.2	46.7	62.1	85.9	56.3	65.5	79.4	55.6	70.0
Tennessee Beardless No. 6 (C. I.[1] 2746)	49.6	20.1	40.5	58.5	27.4	46.8	68.1	36.1	53.0	63.7	35.6	55.9
Esaw	59.9	32.8	54.8	66.5	39.6	59.6	71.9	46.7	65.0	70.4	44.8	63.6

[1] C. I. refers to accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering.

The response to the action of papain is judged from the percentages of free amylase calculated in terms of total amylase (column 3 for each extraction period). A high percentage of free amylase indicates a low response to papain and vice versa. The response is about the same for barley and wheat but considerably less for rye. In accordance with Myrbäck's theory,[5] which is followed in this discussion and in the interpretation of results, this means either that the amylase in

[5] See Introduction.

rye is less bound to the proteins than in wheat and barley or that rye possesses more natural proteinases [6] than do wheat and barley.

TABLE 2.—*Development of total and free diastatic power in degrees Lintner in 4 varieties of dormant wheat during different periods of extraction*

Variety	Diastatic power for extraction period of—								
	3 hours			27 hours			49 hours		
	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total
	°L.	°L.	Percent	°L.	°L.	Percent	°L.	°L.	Percent
Federation	104.1	55.5	53.3	102.1	55.5	54.4	103.0	59.2	57.5
Marquis	116.2	59.2	51.0	115.0	57.8	50.3	112.2	59.5	53.0
Baart	110.0	52.9	48.1	108.8	50.3	46.2	106.2	52.6	49.5
Golden	90.1	44.8	49.7	90.0	43.5	48.4	89.3	44.6	49.9

Variety	Diastatic power for extraction period of—							
	73 hours			97 hours			122 hours	
	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total	Total	Free
	°L.	°L.	Percent	°L.	°L.	Percent	°L.	°L.
Federation	103.0	62.1	60.3	102.0	64.1	62.8	------	64.9
Marquis	110.0	62.9	57.5	113.7	65.4	57.5	------	64.5
Baart	106.2	55.9	52.6	106.2	57.8	57.6	------	57.5
Golden	87.0	46.7	53.7	89.3	48.8	54.7	------	47.6

TABLE 3.—*Development of total and free diastatic power in degrees Lintner in 4 varieties of dormant rye during different periods of extraction*

Variety	Diastatic power for extraction period of—											
	3 hours			27 hours			51 hours			75 hours		
	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total
	°L.	°L.	Percent	°L.	°L.	Percent	°L.	°L.	Percent	°L.	°L.	Percent
Dakold	93.4	71.9	77.1	92.4	76.6	82.9	99.0	83.0	83.8	104.7	85.8	82.0
Star	89.3	70.9	79.4	94.8	76.9	81.1	96.6	82.2	85.5	103.1	86.6	84.0
Rosen	110.5	89.7	81.2	112.2	97.1	86.5	114.2	102.0	89.3	109.4	103.6	94.6
Abruzzi	85.0	65.8	77.4	88.5	71.4	80.7	89.5	77.0	86.0	94.4	82.9	87.8

The four varieties of each barley, wheat, and rye used show a marked variation in diastatic power. They were all grown in the same locality and in the same crop year and the results seem to support Myrbäck's view that variety is a factor in determining diastatic-power differences in cereals.

Dormant oats, corn, and rice (tables 4 and 5) have very little diastatic power. Their total and free diastatic power is about the same, showing that it is not lack of proteinases that is responsible for their low amylase content. Oats seem to be somewhat higher in diastatic

[6] The term "proteinase" as used in this paper includes all natural substances present in biological materials which stimulate, or liberate, amylase.

power than either corn or rice and also show a greater variation between varieties.

TABLE 4.—*Development of total and free diastatic power [1] in degrees Lintner in 4 varieties of dormant oats during different periods of extraction*

| | Diastatic power for duration of extraction period of— | | | | | |
| | 3 hours | | 24 hours | | 48 hours | |
Variety	Total	Free	Total	Free	Total	Free
	° *L.*	° *L.*	° *L.*	° *L.*	° *L.*	° *L.*
Fulghum	3.7	4.5	3.7	4.5	3.7	3.7
Iogold	1.5	1.5	1.5	2.2	2.2	2.2
Lee	1.5	2.2	2.2	2.2	1.5	1.5
Winter Turf	.7	.7	1.5	1.5	2.2	3.0

[1] Corrected for blank values which were about 2.3° L.

TABLE 5.—*Development of total and free diastatic power [1] in degrees Lintner in dormant corn and rice during different periods of extraction*

| | | Diastatic power for extraction period of— | | | |
| | | 3 hours | | 24 hours | |
Cereal	Variety	Total	Free	Total	Free
		° *L.*	° *L.*	° *L.*	° *L.*
Yellow corn	Long-husked Krug	0.7	none	0.7	none
White corn	Thompson Prolific	.7	none	.7	none
Rough rice	Nira	1.5	1.5	.7	none
Brown rice	do	.7	none	.7	none

[1] Corrected for blank values, which were about 2.3° L.

TABLE 6.—*Development of total and free diastatic power [1] in degrees Lintner in dormant buckwheat, soybeans, sorghums, and cowpeas during different periods of extraction*

| | | Diastatic power for extraction period of— | | | |
| | | 3 hours | | 24 hours | |
Material	Variety	Total	Free	Total	Free
		° *L.*	° *L.*	° *L.*	° *L.*
Buckwheat	Silverhull	1.5	1.5	2.2	2.2
Soybeans	Easycook	78.4	78.4		
Do	Rokusun	57.9	58.3	56.5	57.6
Sorghum	Milo	.7	3.0		
Do	Sharon Kafir	.7	.7		
Cowpeas	Brabham	.7	3.0		
Do	Iron	1.5	.7		

[1] Corrected for blank values, which were about 2.3° L.

Of the sorghums and the noncereal seeds (table 6) only the soybeans possess an appreciable diastatic power. Their maximum saccharifying power was developed in 3 hours or less and showed no response to papain, suggesting that the soybeans contain sufficient natural proteinase for the liberation of their saccharifying power. The fact that soybeans are known to be rich in proteinases would seem to support

Myrbäck's theory of the mechanism of liberation of amylase in dormant cereals.

EFFECT OF LACTIC ACID ON LIBERATION OF DIASTATIC POWER

Gőmőry (*18*) used lactic acid as a means of increasing the diastatic power of flour. In the present investigation the effects of lactic acid and papain on the development of diastatic power in barley, wheat, and rye were compared (table 7). The concentration of lactic acid

TABLE 7.—*Effect of lactic acid with and without papain on the development of diastatic power in dormant barley, wheat, and rye during different periods of extraction*

Cereal	Variety	Extractor	Diastatic power for extraction period of—				
			3 hours	26 hours	50 hours	74 hours	98 hours
			°L.	°L.	°L.	°L.	°L.
Barley	Wisconsin Winter	Water	34.5	37.6	46.9	50.8	51.0
		Papain [1]	66.7	69.0	76.4	79.4	77.5
		Lactic acid [2]	17.5	19.0	27.3	31.4	31.4
		Papain + lactic acid	63.7	68.5	73.5	77.5	75.8
Wheat	Federation	Water	55.8	55.8	55.8	59.8	61.4
		Papain	102.0	105.2	104.1	104.1	104.1
		Lactic acid	63.3	72.0	82.6	88.5	89.2
		Papain + lactic acid	103.0	106.2	103.1	104.1	105.2
Rye	Rosen	Water	87.7	93.4	100.0	103.0	103.1
		Papain	111.1	112.2	112.2	113.6	112.2
		Lactic acid	79.4	86.2	93.5	101.0	100.0
		Papain + lactic acid	104.1	110.2	111.1	116.2	112.2

[1] 1/2-percent solution.
[2] 2 cc. of 1-percent lactic acid solution added to the water (60 cc.).

used was 1 percent on the basis of the cereal charges used (the optimum concentration in Gömöry's experiments) or 0.033 percent on the basis of the extracting solution.

There were striking differences in the response of the three cereals to the lactic acid extraction. It definitely increased the diastatic strength of wheat and definitely decreased that of barley. In rye the lactic acid decreased somewhat the diastatic strength in the shorter periods of extraction but had no effect in the longer periods. It had no effect when used together with papain.

The fact that the lactic acid acted differently on the three cereals indicates that its action in increasing the diastatic power of wheat was not a direct hydrolyzing effect. Its different action on barley and wheat, however, may be explained by its effect on the hydrogen-ion concentration of their extracts.

The pH of the distilled water extracts was about 6.5 for wheat and about 5.7 for barley. Balls and Hale (*3*) found that the proteinases of wheat had two optima of digestion, at pH 3.3 and pH 5.0, and that those of barley had a broad pH optimum of digestion from pH 4.0 to 7.0. Accordingly, the pH of the water extracts of wheat was definitely above the pH optima and the pH of the water extracts of barley was right at the optimum. The pH of the lactic acid solution (0.033 percent) used for extraction was 2.90 which is, according to the data of Balls and Hale, close to the lower optimum of digestion for the wheat proteinases but considerably below the optimum for barley. The resultant pH of the lactic acid extracts (in special tests) increased rapidly up to about 3 hours, after which it became about stationary,

namely, pH 4.52 for both the barley and the wheat extracts. This pH is close to the pH optimum of Balls and Hale for wheat, and the increase in diastatic power of wheat caused by lactic acid can be consistently explained by its effect both on the initial and final pH of the extracts. But the final pH of the extracts is also close to the lower end of the broad optimum of digestion for barley mentioned above and therefore could not apparently serve as the cause of the decrease in diastatic activity produced by lactic acid in the barley extracts. However, it is possible that the decrease occurred before the pH of the extract reached its final value. Furthermore, when the data of Balls and Hale (*3, table 2*) are examined more closely, a possible break in the broad pH optimum of proteinase digestion of barley suggests itself at pH 4.50, which is practically identical with the final pH (4.52) of the barley and wheat extracts obtained on the addition of the lactic acid solution.

The results obtained in this experiment, and perhaps also those of Balls and Hale, are not to be viewed as definite values but merely as data showing certain tendencies. This fact, however, should not detract from the plausibility of the explanation offered.

The lactic acid had no effect on the extraction of amylase with papain, probably because the latter was used in large excess. Attention is called to the fact that the increase in the diastatic power caused by lactic acid is not as large as that caused by papain.

EFFECT OF TOLUENE AND MICRO-ORGANISMS ON THE DEVELOPMENT OF DIASTATIC POWER

In the study of the diastatic power of dormant seeds toluene is generally added to prevent microbiological activity. It is important to know whether toluene itself exerts any stimulating or inhibiting influence on the development of diastatic power. It is also of interest to study the effect of the mixed microflora coming from the room atmosphere or from the seeds on the development of diastatic power when its activity is not inhibited by toluene.

In this experiment in addition to ground barley, wheat, and rye, ordinary white wheat flour was used. The results (table 8) show that the use of toluene in water extracts had no effect on rye and only a slight effect on barley but that it had a pronounced depressing effect on the diastatic activity of ground whole wheat and flour.

The fact that when papain was used toluene had no effect on the development of the diastatic power in wheat and flour and also the fact that it had no influence on its development in rye even when no papain was used, tends to show that toluene itself has no direct effect on the development of diastatic power. The depression of the diastatic power in wheat and flour caused by the addition of toluene was evidently due to its inhibiting effect on the growth of micro-organisms.

Jørgensen (*21*) stated that toluene interfered with the depression in the nitrogen solubility of flour caused by the bread improvers—potassium bromate and potassium iodate. The solubility of flour nitrogen, according to Jørgensen, is due to the action of proteinases, similar to the liberation of amylase according to Myrbäck. However on examining closely Jørgensen's data, it is apparent that the depression in nitrogen solubility caused by toluene alone is of about the

same magnitude as that caused by potassium iodate alone. It is possible, therefore, that the solubility of the nitrogen present in the flour was partly due to microbiological activity and that the effect of toluene was to inhibit this activity.

TABLE 8.—*Effects of toluene and papain on the development of diastatic power in dormant barley, wheat, and rye and in ordinary wheat flour*

Material	Variety	Extractor [1]	Diastatic power for extraction period of—			
			3 hours	27 hours	51 hours	72 hours
			°L.	°L.	°L.	°L.
Barley	Wisconsin Winter	Water	31. 2	35. 6	48. 5	49. 2
		Water+toluene	30. 4	32. 4	45. 4	45. 0
		Papain solution	58. 8	63. 7	76. 3	74. 1
		Papain+toluene	58. 8	61. 7	73. 5	73. 5
Wheat	Federation	Water	52. 6	52. 4	86. 2	94. 3
		Water+toluene	54. 8	52. 4	56. 5	57. 5
		Papain solution	104. 1	103. 1	106. 2	104. 1
		Papain+toluene	102. 0	103. 1	106. 2	105. 2
Rye	Rosen	Water	85. 4	90. 0	100. 0	99. 0
		Water+toluene	85. 4	87. 7	100. 0	99. 0
		Papain solution	92. 6	103. 1	113. 7	108. 5
		Papain+toluene	102. 0	108. 7	114. 9	112. 2
Flour		Water	26. 5	25. 5	96. 1	111. 1
		Water+toluene	26. 2	24. 7	25. 0	24. 2
		Papain solution	90. 9	103. 1	119. 0	119. 0
		Papain+toluene	104. 1	102. 0	119. 0	120. 8

[1] Quantity of toluene added, 10 drops.

In ground wheat and in flour the increase in diastatic power caused by microbiological activity was almost as high as that caused by papain. The difference in response to microbiological action between wheat and flour on the one hand and barley and rye on the other is striking (table 8).

DISTRIBUTION OF PROTEINASES AND DIASTATIC POWER IN WHEAT GERM, BRAN, AND FLOUR

The results given in table 8 show also marked differences in the development of diastatic power between ground whole wheat and white flour. With papain or in water without toluene (subject to microbic action) the diastatic power of the flour after 3 days is consistently higher than that of the ground wheat; with toluene or without papain the ground wheat is more than twice as high in diastatic power as the flour. In accordance with Myrbäck's theory whole wheat is therefore richer in active proteinases than flour but poorer in amylase.

To determine whether such is actually the case, bran and germ were each added to flour to the extent of 10 percent of the charges and their effect on the development of diastatic activity of the flour was determined (table 9). This experiment, as well as all other experiments unless otherwise stated, was carried out with the addition of toluene. The flour, germ, and bran were all commercial products but were not obtained from the milling of the same wheat. The Lintner values were corrected for the values of the added bran and germ (free diastatic power, table 10.) The results show that the addition of germ increased markedly the diastatic power of the flour while the addition of bran had but little effect. This tends to show that the proteinases in wheat are largely concentrated in the germ.

Further tests were made by determining the respective response of germ and bran to the added proteinase papain (table 10). It is seen from the percentage recovery of total diastatic power that the bran responded to the papain considerably more than did the germ, indicating that the latter has more natural proteinases than the former. The results also show that both the bran and the germ were lower in total diastatic power than either the ground whole wheat or the flour (table 8), which is in harmony with the fact that flour is higher in total diastatic power than whole wheat.

TABLE 9.—*Effect of addition of 10 percent of wheat bran and wheat germ on the development of diastatic power in flour (duration of extraction 3 hours)*

Material added	Diastatic power		
	Found	Calculated	Increase
	°L.	°L.	°L.
None	27.0		
Germ	44.4	31.1	13.3
Bran	32.2	29.9	2.3

TABLE 10.—*Development of total and free diastatic power in degrees Lintner in wheat bran and wheat germ (duration of extraction 3 hours)*

Material	Diastatic power		
	Total	Free	Ratio of free to total
	°L.	°L.	Percent
Germ	84.0	68.5	81.5
Bran	95.2	56.2	59.0

THE RELATIVE PROTEINASE CONTENT OF VARIOUS SEEDS

The previous experiments have shown that two conditions are necessary for the development of diastatic power in dormant seeds; namely, the presence of amylase and the presence of a stimulating principle which, according to Myrbäck, consists of proteinases.

Some of the seeds tested (tables 4, 5, and 6) failed to develop any appreciable diastatic power, but they also failed to respond to the added proteinase papain. This would tend to show that these seeds are actually lacking in amylase. It was desired to test the various seeds studied for the proteinases responsible for the liberation of amylase. Flour, which is relatively poor in active proteinase but rich in amylase, served as a good testing medium. Accordingly, the seeds studied were added to flour to the extent of 10 percent of the total charges (1.8 gm. of flour and 0.2 gm. of the respective ground seeds) and their effect on the development of the diastatic power was determined.

The results (table 11) show that the amylase content and proteinase content of dormant seeds are not correlated. Barley and rye with an appreciable amylase content have the lowest proteinase content of all seeds studied, while soybeans, also with an appreciable amylase content, have the highest proteinase content. All of the seeds studied which had a low amylase content or none at all showed a relatively high proteinase content, particularly the cowpeas.

TABLE 11.—*Effect of addition of 10 percent of various seeds on the development of diastatic power in flour (duration of extraction 3 hours)*

Ground seed added	Diastatic power		
	Found	Calculated	Increase
	°L.	°L.	Percent
None	26. 0		
Barley	31. 3	27. 4	14. 2
Rye	39. 7	32. 4	22. 5
Oats	42. 4	23. 5	80. 4
Corn, yellow	36. 4	23. 4	55. 6
Corn, white	35. 2	23. 4	50. 4
Rice	37. 6	23. 5	60. 0
Buckwheat	42. 0	23. 5	78. 7
Soybeans	76. 3	31. 2	144. 5
Sorghum	43. 4	23. 7	83. 1
Cowpeas	52. 1	23. 7	119. 8

EFFECT OF SODIUM CHLORIDE ON THE DEVELOPMENT OF DIASTATIC POWER IN CEREALS

The total diastatic power of a plant material in this investigation was determined with the aid of papain. Other proteinases also liberate amylase. Thus Chrzaszcz and Janicki (*10, 11*) found that trypsin and rennet liberated amylase in cereals, while pepsin had no effect. It has been found in this investigation, however, that pepsin liberates amylase in flour.

Since proteinases have a solvent action on proteins, it was desired to determine the effect on the liberation of amylase of an inorganic salt such as sodium chloride which also has a solvent action on protein nitrogen. Chrzaszcz and Janicki (*13*) tested the effect of this salt on ripening wheats and obtained slight increases in saccharifying power. Ford and Guthrie (*17*) obtained increases of saccharifying power in ground barley with potassium chloride.

In this investigation the effect of a 1-percent sodium chloride solution was tested on barley, wheat, rye, and oats. The response of these cereals to this treatment (table 12) showed a marked variation.

The use of sodium chloride resulted in a distinct increase in the diastatic power of the wheat, in a marked decrease in the diastatic power of the barley, and in a small decrease in the diastatic power of the rye. While there was an apparent increase in the diastatic power of the oats, the values involved are too small to indicate the tendency clearly.

The effect of sodium chloride on diastatic power was tested on a few other varieties of wheat (Poole, Fultz, American Banner, Purplestraw) and barley (Mekano Wase and Esaw). The results reported (table 12) for wheat were in a general way corroborated, but those reported for barley were not sustained. It is possible that the effect of sodium chloride on the diastatic power of barley (and perhaps also of the other grains) is a varietal characteristic. Anderson and Ayre (*2*) found that the variation in salt-soluble nitrogen is a varietal characteristic of barley. It is possible that there is a correlation between the effect of sodium chloride on the nitrogen solubility of seeds and its effect on their diastatic power.

TABLE 12.—*Effect of sodium chloride on the development of diastatic power[1] in dormant barley, wheat, rye, and oats*

Cereal	Extractor	Diastatic power for extraction period of—		
		4 hours	24 hours	72 hours
		°L.	°L.	°L.
Barley	Water	40.7	47.5	58.3
	Sodium chloride solution [2]	24.5	28.5	32.8
Wheat	Water	53.2	53.2	59.8
	Sodium chloride solution [2]	75.2	80.3	77.7
Rye	Water	87.4	94.8	101.3
	Sodium chloride solution [2]	83.9	92.1	92.9
Oats	Water	.7	1.5	---------
	Sodium chloride solution [2]	1.5	2.2	2.2

[1] Corrected for blank values which were about 2.3° L.
[2] 1 percent.

Attention is called to the fact that the increase in the diastatic power of wheat caused by sodium chloride (table 12) is less than that caused by papain (table 2).

EFFECT OF PAPAIN ON THE DEVELOPMENT OF DIASTATIC POWER IN FLOUR

Flour, as was shown above, is rich in total amylase and poor in substances which set this amylase free. It could, therefore, serve as an appropriate medium for studying the effect of different activating agents on the liberation of amylase. The effect of various concentrations of papain on the development of diastatic power in flour was studied first.

TABLE 13.—*Effect of various concentrations of papain on the development of diastatic power in flour during different periods of extraction*

Concentration of papain solution	Diastatic power for extraction period of—				
	3 hours	24 hours	48 hours	72 hours	144 hours
Percent	°L.	°L.	°L.	°L.	°L.
None	26.5	25.6	24.6	23.3	---------
0.5	120.5	120.5	---------	---------	---------
.25	120.5	122.0	---------	---------	---------
.10	122.0	119.0	---------	---------	---------
.050	119.0	119.0	---------	---------	---------
.025	117.7	117.7	---------	---------	---------
.010	111.1	117.7	---------	---------	---------
.005	89.3	117.7	119.0	---------	---------
.0025	66.7	85.5	109.0	115.0	120.5
.0010	54.3	86.2	89.3	104.2	116.2
.0005	43.5	55.6	59.2	62.2	72.0

The results (table 13) show that between 0.5 percent and 0.025 percent, the concentration of the papain solution had no effect on the magnitude of the developed diastatic power in 3 hours. The variation in the Lintner values obtained between the limits of these concentrations are to be considered as mere fluctuations in view of the fact that there was no further increase in diastatic power in 24 hours over the corresponding values obtained in 3 hours. Evidently all the concentrations above 0.025 percent were in excess of what was necessary to

liberate the maximum saccharifying power in 3 hours; with the concentrations of papain lower than 0.025 percent the rate of increase in diastatic power was slowed up. With the concentration of 0.0025 percent the maximum saccharifying power was obtained in between 72 and 144 hours. With the lowest concentration the maximum saccharifying power had not been reached after 144 hours when the experiment was discontinued.

Attention is called to the gradual decrease in the free amylase content of the control. The values involved are small but they seem to be consistent, as will be seen in the following experiments.

EFFECT OF PAPAIN ON THE LOAF VOLUME OF BREAD

The theory of Myrbäck and other workers with regard to the development of diastatic power in dormant cereals is completely analogous to the theory of Jørgensen and others (*21, 5*) with regard to the mechanism of the action of bread improvers. The development of free amylase in dormant cereals, according to the one theory, is due to the action of natural proteinases which liberate the amylase by attacking the proteins to which it is bound; added proteinases complete this liberation. The failure of some flours to yield bread of a good loaf volume, according to the other theory, is due to an excess of natural proteinases which weaken the proteins; the bread improvers inhibit the action of these proteinases. By adding relatively large quantities of papain Balls and Hale (*4*) obtained completely collapsed breads.

It was therefore desired to test the effect of the papain concentrations used in the previous experiment (table 13) on the loaf volume of breads obtained with ordinary white flour.

The baking tests were carried out according to the standard procedure of the American Association of Cereal Chemists (*1*). The concentrations of papain (table 14) are given on the basis of the water added (60 cc. of water to 100 gm. of flour). The transverse and longitudinal measurements, as well as the abstract figures representing the products of these respective measurements, give a good idea of the actual gradation in the loaf volume of these breads. There was good correlation between the concentration of papain and the gradation in the loaf volume of the breads. The decrease in volume, as compared with the control loaf, occurred in all except the lowest concentration of papain.[7]

The fact that both the liberation of amylase and the decrease in loaf volume are consistently affected by the concentration of an added proteinase seems to lend mutual support to the two analogous theories. It also seems that the same kind of proteins are involved in the two phenomena. This in turn suggests that only part of the proteins are involved in the liberation of amylase or in the decrease of loaf volume of bread. Such a possibility has indeed been advanced by Myrbäck and Örtenblad (*30*) with regard to the proteins involved in the liberation of amylase, in their controversy with Chrzaszcz and Janicki (*12*). These authors contended that if Myrbäck's theory were true there ought to be a correlation between increased amylase activity and the solubility of protein nitrogen, which, as they found, was not the case. According to Myrbäck and Örtenblad, however, it was not to be

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[7] The effect of papain on the loaf volume of bread was discussed more fully in a previous paper (*15*).

expected that the presumably quantitatively small mass of the amylase substance would be bound to all the proteins; the solubility, therefore, of the correspondingly small portion of the proteins to which the amylase may have been bound could not be detected in the determination of the general solubility of the protein nitrogen.

TABLE 14.—*Effect of various concentrations of papain on the loaf volume of bread*

Concentration of papain solution	Dimensions of loaf		
	Transverse circumference	Longitudinal circumference	Transverse [1] multiplied by longitudinal circumference
Percent	*Inches*	*Inches*	
None	12.8	16.0	205
0.5	9.6	12.6	121
.25	9.8	12.5	122
.125	10.4	13.3	138
.062	10.8	13.8	149
.010	10.7	14.3	153
.005	11.1	14.3	159
.002	11.9	15.3	182
.001	12.5	15.7	196
.0005	12.7	16.1	205

[1] These figures are numerical expressions of the relative volume of the loaves.

EFFECT OF CYSTEINE HYDROCHLORIDE ON THE DEVELOPMENT OF DIASTATIC POWER IN FLOUR

Balls and Hale (4) separated from wheat a partially purified proteinase of the papainase type. This proteinase was activated by cysteine hydrochloride, potassium cyanide, and hydrogen sulfide. In following up the analogy between the theory with regard to bread improvers and the theory with regard to the liberation of amylase by proteinases, the effect of cysteine on the development of the diastatic power in flour was tested.

TABLE 15.—*Effect of cysteine hydrochloride on the development of diastatic power in flour during different periods of extraction*

Concentration of cysteine hydrochloride	Diastatic power for extraction period of—	
	3 hours	24 hours
Percent	° *L.*	° *L.*
None	22.2	22.2
0.3	116.3	120.5
.15	115.0	116.3
.075	96.2	103.1
.037	74.6	80.0

It is shown (table 15) that cysteine hydrochloride has a distinctly stimulating effect on the diastatic activity of the flour and that this stimulation is related to the concentration of the cysteine. The analogy between the two theories is thus sustained.

Much higher concentrations of cysteine than of papain (table 13) were required to produce corresponding stimulating effects on the liberation of amylase. This may be analogous to the results of Balls

and Hale (*5*) who found that relatively large quantities of cysteine hydrochloride were required to complete the dispersion of gluten.

The suggestion made by Balls and Hale (*5*) that cysteine not only serves to stimulate the proteinase but that it also affects the proteins directly, may likewise apply to the stimulation of diastatic activity by this activator. This suggests the possibility that the apparent proteinase deficiency in flour is in a certain degree an activator deficiency which is corrected by such substances as cysteine.

Snider (*36*) found that the addition of cysteine reduced the extraction time of barley malt from 20 hours to 3.

EFFECT OF POTASSIUM IODATE ON THE DIASTATIC POWER OF FLOUR

According to the theory of bread improvers mentioned above, the action of proteinases is inhibited by chemical oxidants, such as potassium bromate and potassium iodate. Jørgensen (*22*) showed that the water-soluble nitrogen in flour was depressed by potassium bromate and potassium iodate. Balls and Hale (*6*) depressed the activity of the proteinase concentrate separated by them from wheat by persulfate, bromate, and metavanadate.

To further test the analogy between the proteinase theories of bread improvement and liberation of amylase, the effect of various concentrations of potassium iodate on the development of diastatic power was studied.

It is shown (table 16) that both the total depression and the rate of depression of the amylase activity are directly related to the concentration of the iodate. Slight depressions of the amylase activity were caused by such low concentrations as a 0.00001 molecular solution of potassium iodate. Table 14 shows that relatively small concentrations of papain caused an appreciable depression in the loaf volume of bread and it was shown elsewhere (*15*) that appropriate concentrations of potassium iodate overcame this depression. The analogy between the two theories is again sustained.

Read and Haas (*32*), in opposing the theory of bread improvers based on the inhibition of proteinases by oxidants, referred to the fact that it takes much more potassium bromate to produce a depression in soluble nitrogen than the quantity of the oxidant required to produce an improvement in loaf volume. Jørgensen (*23*) endeavored to explain this apparent discrepancy. In the present experiments, however, the concentrations of iodate which produced a noticeable depression in the amylase activity of flour are comparable with those used in overcoming the effect of papain in reducing the loaf volume of bread (*15*). It is not claimed that a high amylase content is the cause of decreasing the loaf volume of bread but that the amylase content may, under certain conditions, be an indicator of proteinase activity. When the amylase content is taken as a criterion, not only does the discrepancy pointed out by Read and Haas disappear but there is also, in general, a consistent relation between the concentration of the iodate and the effect produced.

The small quantities of iodate used again suggest that not all the proteins are involved in the two analogous phenomena under discussion but only a small portion of them. Changes in this small portion would not always result in changes in the total nitrogen

solubility which could be detected by ordinary analysis, as was pointed out above.

TABLE 16.—*Effect of various concentrations of potassium iodate on the development of diastatic power in flour during different periods of extraction* [1]

Molal concentration of potassium iodate	Diastatic power for extraction period of—				
	3 hours	24 hours	48 hours	72 hours	144 hours
	°L.	°L.	°L.	°L.	°L.
None	23.2	20.8	19.2	19.2	
0.005	11.3	3.0	3.0		
.0025	14.3	3.8	3.0		
.00125	16.3	5.3	3.8	2.3	
.00062	20.0	8.3	4.5	2.3	
.00016	21.4	13.6	9.8	7.6	6.0
.00008	23.1	17.6	13.5	11.3	9.8
.00004	22.7	18.9	16.3	15.1	12.1
.00002	23.2	20.0	19.5	17.1	11.3
.00001	23.6	21.2	20.0	17.9	
.000005	23.3	21.5	21.4	20.0	

[1] Part of this table was used in a previous paper (*15, table 3*).

The fact that diastatic activity of the flour continually decreased and that, with some of the lower concentrations of the iodate, the maximum decrease had not been reached in 144 hours shows that the oxidant not only inhibits the liberation of the amylase but also inactivates it or destroys it after it is liberated.

EFFECT OF SODIUM CHLORIDE ON THE DEVELOPMENT OF DIASTATIC POWER IN FLOUR

In a previous experiment the effect of sodium chloride on the development of diastatic activity in cereals was tested (table 12). The results were not clearly defined. In this experiment the effect of sodium chloride was tried on flour which, as was pointed out above, serves as a convenient testing material because it is rich in amylase and deficient in proteinases. Salt is also invariably used in bread making. The experiment was carried on in two parts for different intervals of time and with different samples of flour.

Table 17 shows that sodium chloride has a distinctly stimulating effect on the development of diastatic activity and that this effect is directly related to the concentration. As in the case of cysteine, comparatively high concentrations of salt are required to produce the effect and, therefore, it is still a question whether the effect of the salt is on the flour proteinases or directly on the proteins.

The consistent decrease in diastatic activity with time observed in all controls (untreated flours) of the previous experiment (table 13) is sustained in the two controls of this experiment. The same decrease is observed in the two lowest concentrations of salt. This suggests the possibility that there is something in flour, in the nature of the sisto-substances of Chrzaszcz and Janicki (*7, 8*) which has a slightly inactivating effect on amylase. It is possible that sodium chloride, besides its stimulating effect on diastatic activity, also serves as the eleuto-substances of Chrzaszcz and Janicki since this decrease with time is not observed with the concentrations of sodium chloride above 0.025 percent.

TABLE 17.—*Effect of various concentrations of sodium chloride on the development of diastatic power in flour during different periods of extraction*[1]

Part	Concentration of sodium chloride	Diastatic power for extraction period of—			
		3 hours	24 hours	48 hours	72 hours
	Percent	° *L.*	° *L.*	° *L.*	° *L.*
1	None	33.3	29.4	26.7	
	2.0	99.0	104.2	103.1	
	1.0	96.2	100.0	101.1	
	.5	96.2	94.0	93.5	
	.25	86.2	88.5	87.7	
	.10	70.4	70.4	70.9	
2	None	21.1	20.2	19.6	19.7
	.10	66.2	66.2	66.7	66.7
	.05	45.7	45.9	45.9	45.9
	.025	34.6	32.7	30.9	30.9
	.01	26.3	24.0	23.5	23.5

[1] Part of this table was used in a previous paper (*15, table 4*).

To reconcile the decrease in amylase activity after it has been set free, with the proteinase theory, we may assume that the hydrolysis of the protein amylase complex is reversible. The inhibitor or sisto-substance accordingly depresses the proteinase activity, and as a result an equilibrium is established with a lower amylase activity. This is in harmony with the theory of Balls and Hale (*4*) according to which the improvement of the baking quality of some flours with aging is due to the depressed activity of the flour proteinases.

EFFECT OF YEAST ON THE DEVELOPMENT OF DIASTATIC POWER IN FLOUR

When it was found that sodium chloride, an invariable constituent of bread, has a distinctly stimulating effect on the diastatic activity of flour, it was desired to test the effect of yeast, another indispensable constituent of all ordinary bread, on the liberation of amylase in flour. The unidentified microflora ordinarily associated with flour was shown in table 8 to be practically as effective as papain in liberating amylase in flour.

Two concentrations of yeast were used, one which was comparable with bread on the basis of 100 gm. of flour used in the baking of the "small" loaf according to the procedure of the American Association of Cereal Chemists (0.06 gm. yeast per 2 gm. flour and 60 ml. water), and one comparable with bread on the basis of water used (3 gm. yeast per 2 gm. flour and 60 ml. water). The same volume of water (60 ml.) used in the preparation of the small loaf was used for the extraction of amylase in these experiments. The tests with yeast were made with and without toluene.

Table 18 shows that when no toluene was added even the low concentrations of yeast caused a distinct increase in diastatic activity as compared with the control to which toluene was added. However, neither the low nor the high concentration of yeast was as effective in stimulating the diastatic activity of flour as the unidentified microflora associated with flour when its action is not suppressed by toluene (table 8). The probable explanation is that the yeast suppressed the activity of the mixed microflora of the flour and that the former is not as effective in stimulating diastatic activity as the latter.

TABLE 18.—*Effect of yeast on the development of diastatic power in flour during different periods of extraction*

Material	Yeast added	Diastatic power for extraction period of--	
		3 hours	72 hours
	Grams	° *L.*	° *L.*
Flour	{0+toluene	26.9	24.3
	.06 yeast only	35.9	97.0
	.06+toluene	31.0	32.4
	3.0 yeast only	94.3	111.1
	3.0+toluene	88.5	108.8
No flour	3.0 yeast only	0	0

This explanation is supported by the fact that the high concentration of yeast used caused practically the same stimulation of diastatic activity in flour with and without the addition of toluene which suppressed microbic activity in the previous experiments. Toluene also practically suppressed the activity of the lower concentration of yeast in this experiment but was ineffective against the higher yeast concentration. The yeast itself had no diastatic power.

The fact that two indispensable ingredients of bread cause a marked increase in the diastatic activity of flour (salt and yeast caused a distinct increase in the diastatic power of dough also) tends to show that the depression in volume of bread resulting from the addition of certain concentrations of papain and other proteolytic enzymes is not due directly to the increased amylase content of the flour caused by these enzymes but to some other factor, probably to the degradation of the proteins. The increase in amylase activity would accordingly serve only as an indicator of proteinase activity. The relation of free amylase activity to total amylase activity in flours should be indicative, in a general way, of their relative proteinase strength.

EFFECT OF YEAST ON THE DIASTATIC ACTIVITY OF CEREALS

It was shown in a previous experiment (table 8) that mixed unidentified microflora affected variously the diastatic activity of barley, wheat, rye, and wheat flour. The effect of the microflora on the ground wheat and the wheat flour was most pronounced. The stimulating effect of yeast (table 18) on the diastatic activity of flour was also very pronounced.

To study further the comparison between the mixed unidentified microflora and yeast the effect of the latter on the diastatic activity of barley, wheat, and rye was tested. Three grams of yeast were used and no toluene was added to any extractions, as the previous experiment had shown that with this quantity of yeast toluene had little effect. However, toluene was added to the controls.

As indicated in table 19, the yeast stimulated appreciably the diastatic activity of the wheat, depressed to nearly one-half that of barley, and only slightly depressed that of rye. When the effect of the yeast is compared with that of the mixed unidentified flora (table 8) it is seen that the wheat was affected in about the same way, that there was but a slight difference in the effects on rye, but that there was a pronounced difference in the effects on barley; the unidentified micro-

flora caused a slight increase in the diastatic activity of the barley, while the yeast caused a pronounced decrease. It should be noted, however, that while the same varieties of wheat and rye were used in the two parallel tests (tables 8 and 19) the varieties of barley were different. The different response of the two barleys may, therefore, have been due to varietal characteristics.

TABLE 19.—*Effect of yeast on the development of diastatic power in dormant barley, wheat, and rye during different periods of extraction*

Cereal	Variety	Added yeast	Diastatic power for extraction period of —	
			3 hours	24 hours
		Grams	°*L.*	°*L.*
Barley	Tennessee Winter	None	40.1	46.7
		3.0	23.7	22.3
Wheat	Federation	None	55.5	55.5
		3.0	54.1	74.1
Rye	Rosen	None	89.7	97.1
		3.0	74.6	87.6

TOTAL DIASTATIC POWER OF DORMANT CEREAL GRAINS AND FREE DIASTATIC POWER OF MALTS

It was formerly believed that the diastatic activity of barley malts was due to enzymes synthesized during germination in the malting process. The present view is that the amylase is preexistent in the barley grains but that it is liberated or activated during germination (26). The liberation of amylase in malt is consequently analogous to the liberation of total amylase in dormant seeds except that in the former it is accomplished by proteinases or activators naturally developed in the seed during germination. The question consequently arose how the total diastatic activity of dormant seeds obtained by means of papain compared with that of malts. Syniewski (37) found that barley extracted with a papain solution had a higher saccharifying power than the water extract of the corresponding barley malt. More recently Sallans and Anderson (33) have also reported results which show the total diastatic activity of dormant barleys to be much higher than that of the corresponding malts. On the other hand, Hills and Bailey (19) and Chrzaszcz and Janicki (12) found that the total diastatic activity of dormant barley is lower than the free diastatic activity of the corresponding malts.

In this investigation also the total diastatic power of the cereals studied was compared with that of their corresponding malts. The experimental malts were prepared as follows: 10 gm. of grain, placed in a Petri dish between two layers of filter paper, were wetted with 10 cc. of distilled water, covered, and allowed to stand at 50° F. for 6 days. The germinated cereals were then dried, freed from rootlets and plumules, and ground. The diastatic power of the malts prepared in this way compared well with corresponding malts prepared in the standard way.

It is seen (table 20) that in a general way the total diastatic power of the dormant cereals is correlated with the free diastatic power of the corresponding malts, but that, with the exception of two cases in

which the difference was very small, the free diastatic activity of the malts was appreciably higher than the total diastatic power of the dormant seeds. The results corroborate those of Chrzaszcz and Janicki and Hills and Bailey.

The extent of the increase in diastatic activity of the malts over the total diastatic activity of the dormant seed varies and may be a varietal characteristic.

Hills and Bailey are of the opinion that the increase in saccharifying activity of malts might be due to the alpha-amylase which develops during germination. The striking increase in saccharifying power of the germinated oats may be due entirely to alpha-amylase since the dormant oats are deficient in saccharifying power notwithstanding the fact that they are comparatively rich in proteinases, and since their saccharifying power did not increase when papain was added.

EFFECT OF PAPAIN ON THE DIASTATIC POWER OF MALTS

Hills and Bailey (*19*) came to the conclusion that the beta-amylase content of barley does not increase during the malting process. Papain which increases the saccharifying power of cereals, presumably due to their increase in beta-amylase content, ought accordingly to increase the saccharifying power of malts. Indeed Hills and Bailey found that the addition of papain did increase the saccharifying power of green malts.

In this investigation the total and free saccharifying power of the dry malts obtained from the cereals studied were compared. The experimental malts of the preceding experiment were used in this test.

TABLE 20.—*Total diastatic power in degrees Lintner of different varieties of dormant barley, wheat, rye, and oats compared with the free diastatic power of the corresponding malts*

Cereal	Variety	Free diastatic power in malts	Total diastatic power in dormant seeds	
			Diastatic power	Ratio of dormant seeds to malt
		°*L.*	°*L.*	*Percent*
Barley	Wisconsin Winter	104.7	88.5	84.5
	Tennessee Winter	93.5	79.4	84.8
	Tennessee Beardless No. 6 (C. I. 2746)	65.8	63.7	97.0
Wheat	Federation	126.5	102.0	80.7
	Marquis	160.0	113.7	71.0
	Baart	125.0	106.2	85.2
	Golden	90.9	89.3	90.4
Rye	Dakold	155.0	104.7	67.5
	Star	128.2	103.1	80.6
	Rosen	132.4	109.4	82.7
	Abruzzi	118.4	94.4	79.6
Oats	Fulghum	20.6	3.7	17.9
	Iogold	23.5	2.2	9.4
	Lee	17.7	1.5	8.5
	Winter Turf	19.5	3.0	15.4

It is seen (table 21) that the papain increased the saccharifying power of all malts except those of the oats; in these the papain caused a slight depression in diastatic activity similar to that caused in the dormant oats (table 4). The behavior of the oat malts in this respect

would lend support to the theory that the increase in saccharifying activity of malts caused by papain is due largely to an increase in beta-amylase in which oats seem to be deficient.

TABLE 21.—*Total and free diastatic power in degrees Lintner of malts obtained from different varieties of barley, wheat, rye, and oats*

Cereal	Variety	Diastatic power		
		Total	Free	Ratio of free to total
		°L.	°L.	*Percent*
Barley	Wisconsin Winter	92.2	83.0	90
	Tennessee Winter	80.6	70.2	87
	Tennessee Beardless No. 6 (C. I. 2746)	58.8	48.1	82
Wheat	Federation	132.4	119.8	90
	Marquis	170.9	138.9	81
	Baart	144.9	104.7	72
	Golden	93.0	77.8	84
Rye	Dakold	137.9	129.0	94
	Star	122.0	118.3	97
	Rosen	127.4	105.8	83
	Abruzzi	104.2	99.0	95
Oats	Fulghum	24.2	25.6	106
	Iogold	22.6	25.6	113
	Lee	16.6	18.1	109
	Winter Turf	16.6	18.1	109

TABLE 22.—*Effect of age on the free diastatic power of malts obtained from different varieties of barley, wheat, rye, and oats (duration of extraction 3 hours)*

Cereal	Variety	Free diastatic power		
		Fresh malt [1]	Malt 8 to 15 months old	Ratio of old to fresh malt
		°L.	°L.	*Percent*
Barley	Wisconsin Winter	104.7	83.0	79
	Tennessee Winter	93.5	70.2	75
	Tennessee Beardless No. 6 (C. I. 2746)	65.8	48.1	75
Wheat	Federation	126.5	119.8	95
	Marquis	160.0	138.9	87
	Baart	125.0	104.7	84
	Golden	90.9	77.8	86
Rye	Dakold	155.0	129.0	83
	Star	128.2	118.5	92
	Rosen	132.4	105.8	80
	Abruzzi	118.4	99.0	84
Oats	Fulghum	20.6	25.6	124
	Iogold	23.5	25.6	109
	Lee	17.7	18.1	102
	Winter Turf	19.5	18.1	93

[1] Barley and rye malts were prepared in March 1938, wheat malts in September 1938, and oat malts in November 1938.

The increases in saccharifying power caused by papain in the malts of barley, wheat, and rye are consistently lower than those caused by papain in the corresponding dormant seeds (tables 1 to 3). This may be due to the fact that the malts used in these tests were from 8 to 15 months old, and it is shown (table 22) that these malts, again with the exception of the oats, sustained appreciable losses in saccharifying power on aging. These losses may have been sustained largely at the

expense of the beta-amylase content. It was shown above that the saccharifying power of flour extracts, which is presumably due almost exclusively to beta-amylase, also decreases gradually on standing.

The increases in saccharifying power caused by papain in the dormant seeds of the four varieties of barley studied (table 1) compare very well with those reported by Hills and Bailey (*19*) for the green malts of several barley varieties, especially for the 1935 crop. The same authors have shown that their dry malts were lower in saccharifying power than the corresponding green malts. These two facts seem to support the explanation offered above that the discrepancy in this investigation between the respective increases caused by papain in the saccharifying power of the malts and of the corresponding dormant seeds was due to the loss of beta-amylase from the malts during storing.

DISCUSSION

Barley, wheat, and rye show differences in "free" and "total" amylase content between one variety and another in the same species and even more so between one species and another.

The differences between barley, wheat, and rye on the one hand and oats, corn, and rice on the other is particularly striking. It is remarkable that, since the early history of its planned economy, the white race has selected as its staff of life barley, wheat, and rye which have a relative abundance of free and total amylase.

Barley, wheat, and rye differ among themselves not only in their content of total and free amylase but also in the response of their respective diastatic activity to activation by various agents. This was shown particularly by the diverse effect of the acidity of the medium, of the unidentified microflora as well as of yeast, on the diastatic activity of wheat and barley.

While no conclusion is justified from these results regarding the role of variety in the differences of the amylase content, since they represent only 1 year's results, they nevertheless support the already overwhelming evidence that differences in amylase content represent varietal differences. Some observations made in this investigation on differences between varieties of the same cereal with regard to their amylase activity, if sustained, suggest the possibility that varietal differences which could not be detected by ordinary analytical methods are to be looked for in this biological field. Varieties may differ not only with respect to total and free amylase but also with respect to the quantitative relation of the two kinds of amylase (alpha and beta), to the way in which their diastatic activity is stimulated, to their proteinase activity as indicated by their effect on a medium such as flour which is rich in amylase but poor in active proteinase, and with respect to the optimum pH digestion of their proteinases. These differences may be responsible for the differences in the baking quality of the flour produced from different varieties of wheat. Differences in the optima of digestion of the proteinases may perhaps account for the fact that one variety of a cereal is sometimes more affected by the acidity of a soil than another variety of the same cereal.

In the group of the sorghums and noncereal seeds there is a striking difference between soybeans, which have a relative abundance of amylase, and the other seeds which have little or no amylase. The

presence of a relatively large quantity of amylase in soybeans is somewhat surprising since they are practically devoid of starch. It would seem, therefore, that amylase has some other function in plant metabolism besides the transformation of starch into sugar.

The results obtained in this investigation also throw some light on the mechanism of stimulation of amylase activity in seeds. The analogy between the theory that the so-called bread improvers inhibit the excessive proteolysis of the flour proteins by natural proteinases found in flour and the theory that amylase in cereals is combined with proteins and is set free by proteinases naturally found in the cereals or added proteinases, is clearly brought out. These two theories lend mutual support to each other. Added proteinases like papain, pepsin, etc., which decrease the loaf volume of bread also liberate amylase in flour. Chemical oxidants which increase the loaf volume of bread, presumably by inhibiting the flour proteinases, also depress the diastatic activity of the flour.

The analogy between the two theories is also strengthened by the identical arguments made against them by their respective opponents. Chrzaszcz and Janicki, who oppose the theory of amylase liberation of Myrbäck, and Read and Haas, who oppose the theory of Jørgensen, claim respectively that the increase diastatic activity caused by proteinases is not commensurate with the increase in nitrogen solubility in the case of the former theory and that the depression in nitrogen solubility is not commensurate with the increase in the loaf volume of bread in case of the latter theory. The answer to the respective arguments, as was pointed out in the discussion of the corresponding individual experiments, is also identical: not all the proteins are involved in the analogous phenomena covered by the two theories but only a portion of them. When the increase in diastatic activity caused by proteinases, such as papain, is correlated with their effect in decreasing the loaf volume of bread or when the increase in loaf volume of bread caused by bread improvers such as potassium iodate is correlated with their effect in depressing diastatic activity there is a direct relation between the concentration of the causal agents and their effect in the two sets of phenomena.

While there is a distinct correlation between decrease of loaf volume and stimulation of diastatic activity caused respectively by proteinases, such as papain, and by bread improvers, such as potassium iodate, there is evidence which tends to show that the changes in amylase activity referred to are not directly responsible for the corresponding changes in loaf volume. The amylase changes serve only as indicators of some other changes, probably of changes in the proteins due to the activity of the proteinases which liberate the amylase.

It was shown that several of the seeds studied are poor in amylase but possess appreciable proteinase activity, which was demonstrated by their effect in stimulating the diastatic activity of flour. This suggests a possible new method for the determinztion of the gross proteolytic activity which might, in certain instances, take the place of the more laborious nitrogen-solubility, viscosity, or titration methods. Similarly, the quantitative relation between free and total amylase of a biological material may also serve under certain conditions as an indication of the proteinase strength of these materials,

especially of proteinases which are factors in determining the baking qualities of flour.

While most of the facts and observations brought out in this investigation favor the proteolytic mechanisms of amylase liberation suggested by Myrbäck and others, the effect of the nonproteinase agents on the liberation of amylase could be interpreted as lending some support to the theory of sisto-substances and eleuto-substances of Chrzaszcz and Janicki. Thus cysteine and sodium chloride could be considered as eleuto-substances while the depressing effect of aging on the amylase activity of flour could be attributed to a sisto-substance. The character of activity of the nonproteinase agents varies. Cysteine, for instance, like the proteinases, stimulates diastatic activity and depresses the loaf volume of bread; the effect of aging is similar to that of a bread improver; it depresses diastatic activity and increases the loaf volume of bread. On the other hand, sodium chloride and micro-organisms stimulate diastatic activity without decreasing the loaf volume of bread to the same extent as the proteinases. There is still therefore the question whether the nonprotein agents act directly on the amylase and proteins or on the proteinases. Balls and Hale came to the conclusion that cysteine acts on the proteins and that the effect of aging is connected with proteinase activity. It is possible that the stimulating effect of peptone on diastatic activity which is Chrzaszcz and Janicki's strongest proof in favor of the existence of eleuto-substances, is in reality due to its effect on microbiological activity.

SUMMARY

Dormant seeds of barley, wheat, rye, oats, corn, rice, buckwheat, soybeans, sorghums, and cowpeas and also ordinary white flour were analyzed for total and free amylase.

Of the principal cereals, wheat showed the highest diastatic activity; rye was close to wheat and barley was considerably below wheat; oats, corn, and rice showed very little diastatic activity.

Of the other seeds studied soybeans showed a relatively high diastatic power; the buckwheat, cowpeas, and sorghums had a very low saccharifying power or none.

Each of the four varieties of barley, wheat, and rye showed a variation in the amylase content. This variation was consistent for the total as well as for the free amylase.

The diastatic activity developed in barley, wheat, and rye with the addition of papain (total amylase) was invariably higher than that developed without the addition of papain (free amylase). Papain had no effect on the saccharifying power of soybeans nor on that of the seeds which had a low free amylase content.

Ordinary white wheat flour showed a much lower free diastatic power and a higher total diastatic power than ground whole wheat.

Baker's yeast and certain common unidentified microflora increased the diastatic power of ground whole wheat and flour but had little effect on rye and oats; baker's yeast decreased the diastatic power of barley but the microflora had little effect on it.

Lactic acid increased the diastatic power of flour and ground wheat and depressed that of barley. There is a possibility that the effect of lactic acid is due to a shift in the pH of the respective extracts. In

the case of wheat this shift is toward the optimum digestion of the wheat proteins; in the case of barley it is away from that optimum.

Sodium chloride increased the diastatic power of wheat and flour and decreased that of barley and rye. In flour the liberation of amylase was related to the concentration of the salt.

There is evidence that the effect of lactic acid, microbiological activity, and sodium chloride on the diastatic activity of the cereals varies with the variety of the cereal.

There is evidence supporting the theory on the mechanism of liberation of amylase in cereals advanced by Myrbäck and others; namely, that the amylase is combined with proteins and is liberated by proteinases naturally found in cereals or by added proteinases.

A clear analogy is established between the theory with reference to the mechanism of the liberation of amylase in cereals by proteinase activity and the theory with reference to mechanism of action of the bread improvers; namely, that the chemical oxidants inhibit the degradation of the flour proteins by proteinases naturally found in it. There is evidence that the same kind of proteins and proteinases are involved in the two analogous phenomena. The two theories mutually support each other.

The decrease of the loaf volume of bread and the liberation of amylase in flour were respectively correlated with the same concentrations of papain.

Potassium iodate, which is used as a bread improver, depressed the diastatic power of flour; the depression was correlated with the concentration of the iodate.

Cysteine, like sodium chloride, stimulated the diastatic activity in flour, the stimulation being related to the concentration of the stimulant. However, cysteine decreases the loaf volume of bread, while sodium chloride does not, which shows that there is some difference in the mode of action between these two nonproteinase stimulants of diastatic activity.

Flour was found to be rich in amylase and poor in active proteinases. It was also found to be a good medium for testing the effect of proteinases and for the estimation of proteinases in other plant materials.

By using flour as the testing material it was found that the proteinase content in plant materials was not correlated with their amylase content. Barley and rye with a relatively high amylase content showed the lowest proteinase content of all the seeds studied. Of the principal cereals, oats, which have a relatively low amylase content, had the highest proteinase content. Of all seeds studied soybeans, with a high amylase content, had also the highest proteinase content. The next highest proteinase content was that of cowpeas, which have a low amylase content. Wheat germ and bran have a lower total amylase content and higher proteinase content than flour. The germ had a higher proteinase content than the bran.

The free diastatic power of the malts prepared from cereals was higher than the total diastatic power of the same dormant cereals. Papain increased the diastatic power of these malts.

The experimental malts prepared from barley, wheat, and rye lost part of their diastatic strength with aging.

Apparently the increase in free diastatic power of malts over that of the corresponding cereals is due largely to an increase in alpha-amylase.

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PATHOLOGICAL HISTOLOGY OF SUGARCANE AFFECTED WITH CHLOROTIC STREAK [1]

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INTRODUCTION

Chlorotic streak is one of the least understood diseases of sugarcane. Potentially it is of considerable economic importance, since it is known to cause a marked reduction in the germination of plant cane and ratoons; this effect, together with the later stunting of growth, results in severe losses in tonnage. Such losses have occurred in localized areas of Louisiana, where infection reached a fairly high level before control measures were instituted. The history of the discovery and relatively rapid spread of the disease in that State conforms to the pattern for virus diseases, and the proof that it is transmitted by the leafhopper *Draeculacephala portola* (Ball) [2] not only throws light on the spread of the disease but also gives strong indication that it is caused by a virus. However, in earlier work Carpenter [3] interpreted certain bodies found in association with chlorotic streak as fungi. In the present study of the pathological histology of the disease an opportunity was afforded to search again for organisms that might be associated with it.

The most reliable diagnostic symptom of the disease is the appearance on the leaves of chlorotic streaks, which vary in length from a few millimeters to nearly the entire length of the leaf. The streaks are distinguished from other streaks occurring on sugarcane leaves by their broken, wavy, irregular margins. Abnormal stiffness and straightness of the foliage, especially of young plants, and reddening of vascular bundles in the stalks, particularly in the nodal region, frequently occur in diseased plants. However, these symptoms have not been sufficiently differentiated from similar effects produced by other causes to be relied upon for positive identification of chlorotic streak in the absence of the characteristic streaks.

Chlorotic streak apparently is not a completely systemic disease. All aerial parts of the plant may be affected to some degree at one time or another, but there is evidence that many plants are not completely invaded, and, even after the disease has developed, apparently complete recovery may occur.[4] In a disease of such erratic behavior, it is particularly desirable to ascertain the sequence in its histological development with respect to the tissues affected. This study of the pathological histology of sugarcane affected with the disease was undertaken as an aid to a better understanding of its nature and its effects on the plant.

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MATERIALS AND METHODS

The C. P. 29/320 variety of sugarcane was used in most of this work. Material was taken from the dormant and sprouting axillary buds, young shoots, old stems, and leaves of diseased and healthy plants.

The principal killing fluids used were formalin-acetic-alcohol (F. A. A.), Bouin, and various modifications of Nawashin's formula (Craf). Although all of these yielded generally satisfactory sections, the Craf formulas gave the best preservation of cells. For basic fixation the Erlicki-Zirkle formula was used.

The dehydrants and solvents used in various combinations were acetone, ethyl alcohol, ethyl ether, chloroform, tertiary butyl alcohol, anhydrous dioxane, and xylol. Progressive infiltration in Parowax was followed by casting in commercial Tissuemat or in a similar matrix compounded with gum rubber, beeswax, and paraffin. The hot-celloidin and cold-celloidin methods were also employed.

Of the various stains and combinations used, iron-alum haematoxylin and safranine and fast green were preferred. The specific methods used are indicated in the text and in some of the explanations of plates.

HISTOLOGICAL STUDIES

BUD

Axillary buds were taken at random from mature, field-grown, diseased stalks and embedded as just described. Sections from most of these showed no histological abnormalities when compared with healthy material. Some of these buds may not have been invaded by the disease, since it is known that buds on diseased stalks may give rise to apparently healthy plants. However, from the large number of buds studied and their random selection from many stalks, it may be assumed that at least some of them were invaded and would have produced diseased plants.

In some buds there were marked pathological disturbances. Several or all of the differentiating leaves showed necrosis in varying degrees, and in some leaves large necrotic cavities had developed (pl. 1, *A*, *B*). Necrosis was accompanied by the abundant production of a dark-staining gum. The gum filled the cavities as an amorphous mass or was in the form of spherical globules of varying size, some of which assumed a pelliclelike covering resembling a wall. The presence of these spheres in a necrotic strand is shown in plate 1, *C*, *D*. Some are teardrop-shaped; others are rounded and have a wall-like covering.

Frequently the necrosis was initiated in the epidermis of the adaxial surface of the differentiating leaves, and sometimes it extended for some distance along the epidermal layer with only slight penetration into the mesophyll (pl. 2, *A*). Often, however, the mesophyll was involved along with the epidermis, and in some leaves the necrotic condition was limited to the parenchyma.

It is improbable that a bud showing as severe necrosis as that illustrated in plate 2 would germinate, and since one of the effects of chlorotic streak is the suppression of germination of the buds on planted cuttings and ratoons, it may be assumed that the failure to germinate is at least partly the result of the necrotic condition described. On several occasions, buds from diseased stalks that had
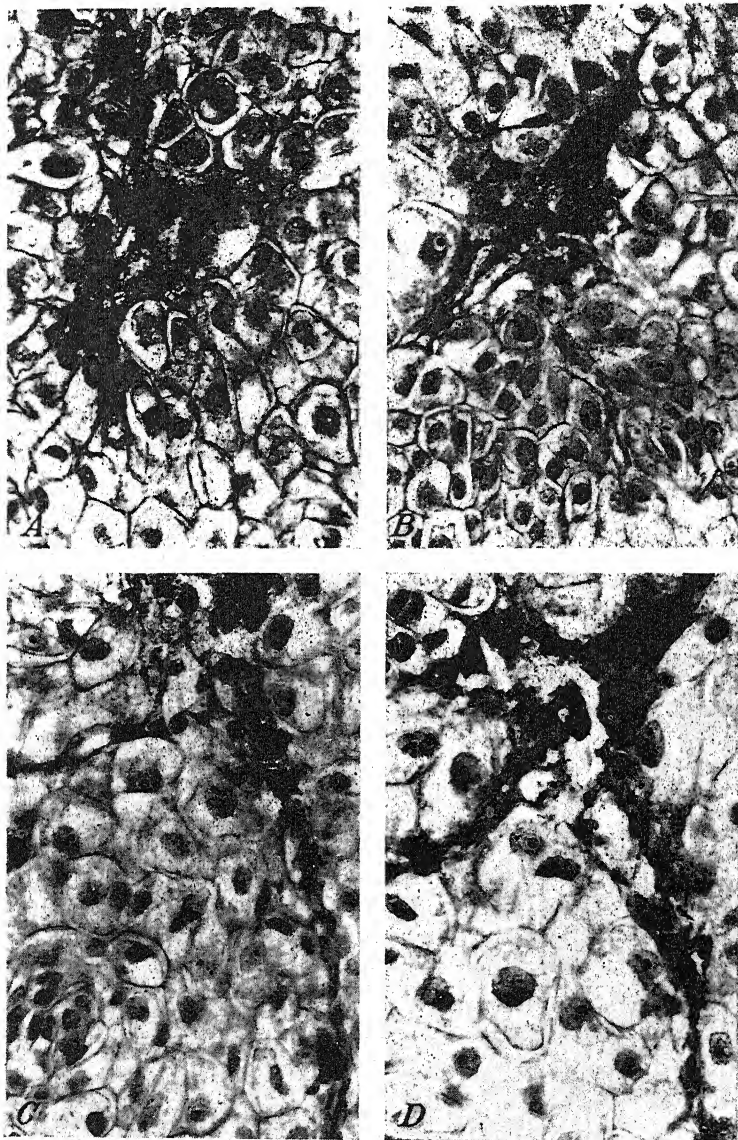
Necrosis in diseased axillary buds. *A*, *B*, Necrotic cavities developing in the mesophyll. ×400. *C*, *D*, Necrotic strands radiating from cavities in the leaf parenchyma of ungerminated buds, with spherical globules appearing in the mass of gum. ×400.
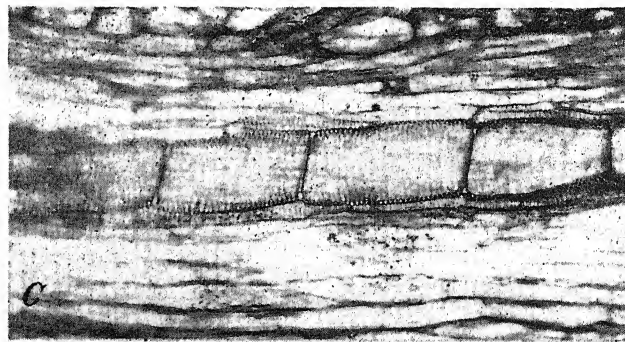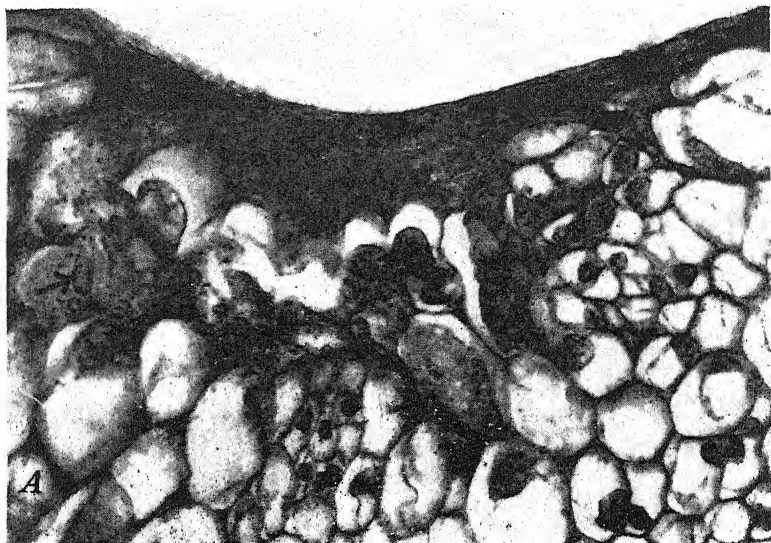
A, Necrotic area in a diseased bud, initiated in the epidermis and extending into
parenchyma; arrow indicates plasmodiumlike extrusion from one cell into
another. ×800. B, Unstained fresh section of stem tissue prior to dehy-
dration, showing clumps of a translucent, granular substance resembling
plasmodiumlike bodies. ×800. C, The section shown in B, after dehy-
dration in dioxane; note the almost complete dissolution of the substance.
×800. Similar dissolution occurred in several fat solvents, particularly in
xylol.

failed to germinate after several days' incubation at 35° to 36° C., which is optimum for germination of sugarcane, were found, on sectioning, to have necrotic areas.

LEAF

A histological study was made of the progressive development of the leaf streaks from their incipiency to necrosis. Stained sections of embedded material were used for the study. The streaks may make their first appearance on a leaf at any stage of development, from the time it unfolds from the spindle until senescence. At first the streaks are very faint chlorotic lines with irregular margins, but they may increase rapidly in size until they extend most of the length of the leaf and may broaden to approximately half an inch in width. Within as few as 7 days the centers of the streaks may become necrotic. Frequently, however, the streaks enlarge very little from their original size, remaining small and faint throughout the life of the leaf; and many of the streaks that do increase in size do not become necrotic. The streaks retain the characteristic irregular margins, regardless of the size they attain.

Leaves of germinating buds and of young shoots were studied to determine whether incipient streaks could be detected prior to the unfolding of the leaf. In the material studied, plastid development appeared to be normal and no marked tissue abnormalities were recognized at this stage.

Some leaves on diseased plants, particularly the first leaves produced, do not develop well-defined streaks but have an over-all "scalded" appearance, with poorly defined chlorotic areas. Frequently the tips and margins of the leaves become dried. In the field, this is particularly noticeable during prolonged dry weather in the spring, when the plants in areas of high infection may have a scorched appearance.

In these "scalded" leaves, as in the prominently streaked areas of other leaves, the chloroplasts are markedly reduced in number, size, and stainability. In some cells of the mesophyll the protoplast may be clumped into a dark-staining mass. Thickening of the walls of mesophyll and epidermal cells, a process that also precedes necrosis in the streaked areas, may occur. Similar histological disturbances may develop in the bundles. Walls of either phloem or xylem, or both, may be abnormally thickened, and both may show various amounts of a gumlike deposit. It is only in the "scalded" leaves that an abnormal condition of the phloem has been observed in advance of necrosis in the mesophyll.

The streaks may appear on only one or on several leaves of a diseased plant. In Louisiana it is comparatively rare for all the leaves of a plant to exhibit the symptoms at one time, and there may be periods in the life of a diseased plant during which it is symptomless so far as the leaf streaks are concerned.

Chloroplasts in the incipient streaks show a distinct reduction in number and size, a loss of stainability, and pronounced clumping as compared with chloroplasts in the adjacent tissues. Frequently the thickness of the streaked portion of the leaf is also reduced, and there is a tendency toward excessive brittleness of these areas when micro-

tomed. As the streaks increase in size, there is an extension of the area with reduced chloroplasts.

Necrosis begins with the disorganization of the protoplast, culminates as a rule in the darkening and dissolution of the cell wall, and is accompanied by a heavy deposition of gum. In fixed material the entire cell contents may be clumped into a dark-staining mass (pl. 3, *E*). Usually the mesophyll or the border parenchyma is affected in advance of the vascular tissue, although in some instances bundles showing marked necrosis are surrounded by apparently normal tissue, except for the reduction in the number and size of chloroplasts.

As necrosis proceeds, the cell walls become wrinkled in outline and presently collapse, but as a rule they do not rupture until necrosis is far advanced. Splitting of the leaf along the necrotic streak may occur, although the streak may remain intact during the life of the leaf.

In the affected cells of necrotic leaf tissue there is usually profuse gummosis. The gum is apparently of the same nature as that occurring in the stems. Some of the cell inclusions consist of spherical globules of varying size, some of which may develop a pelliclelike outer layer resembling a wall (pl. 3, *E*, *F*). Some of these bodies occur in apparently normal tissues of the diseased leaf. Regardless of size and of their apparent resemblance to definite organized bodies, however, the contents of these bodies have been found to be uniformly homogeneous, with no evidence of nuclear structure.

Other intracellular inclusions, the origin of which is less obvious, occur in the diseased leaves. Two of these in a bulliform cell are shown in plate 3, *C*.

STEM

Several hundred single-bud cuttings were made from both diseased and healthy stalks; the latter were treated with hot water at 52° C. for 20 minutes to insure freedom from the disease, and were germinated in steamed soil in the greenhouse. Material for embedding was taken at the time of emergence of the shoot from the soil, when the shoots were 6 to 8 inches tall from the dewlap, and again when they were 12 to 18 inches tall. At this last stage no histologically mature internodes had emerged above soil level.

Tissues of the growing point and young internodes of diseased shoots appeared to be normal. Evidence of the presence of the disease was not found until internodes of the shoot approached histological maturity. In these internodes necrotic strands in the pith parenchyma were observed, in some instances involving only a few cells, but in others extending for some distance through the parenchyma. Necrosis was also found sporadically in the pith parenchyma of mature field-grown stalks (pl. 3, *A*, *B*), in which a partial or complete dissolution of the cell wall occurred. In regions having extensive necrosis of parenchyma, the bundles remained as islands of apparently normal vascular tissue enclosed in necrotic parenchyma. This is similar to the pattern of necrosis observed by Sartoris [5] in stem necrosis of nonparasitic origin and suggests that the development of necrosis may follow the same general course regardless of cause. Un-

[5] Sartoris, G. B. Necrotic stripes in sugarcane. Jour. Hered. 31: 515–520, illus. 1940.
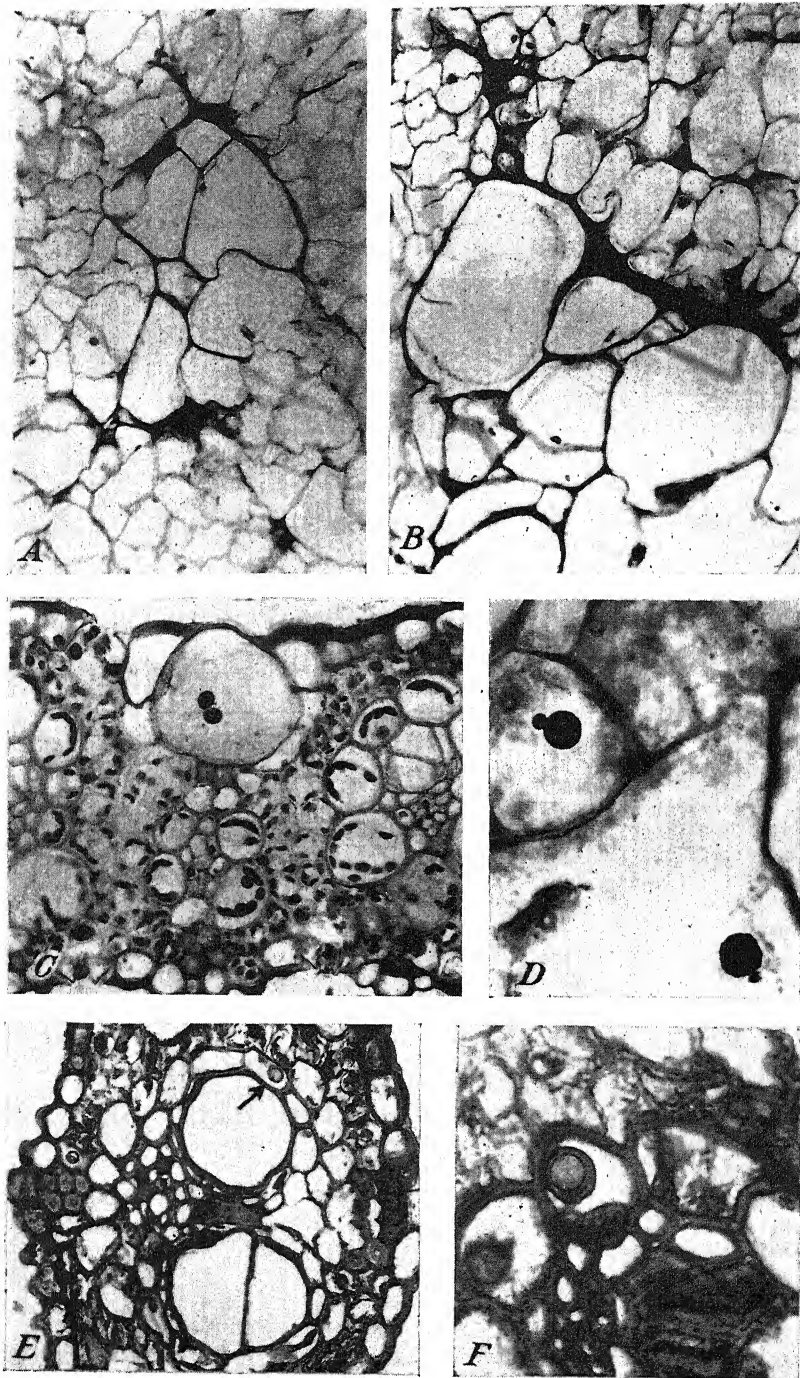
A, B, Necrosis of parenchyma of mature, field-grown stalks.   ×900.   C, Spherical
bodies in a bulliform cell of a diseased leaf at some distance from a streak.
×320.   D, Dark-staining, spherical bodies in the nodal region of young dis-
eased shoots.   ×900.   E, Necrosis in leaf tissue, showing collapse of the
mesophyll cells surrounding a large bundle; the sheath cells are also collaps-
ing, and deposit of gum is shown in the conductive elements; arrow indicates
a spherical "walled" body.   ×400.   F, Enlargement of

like the condition found in the leaf, general necrosis of stem bundles was not observed.

Necrosis is associated with the production of abundant deposits of materials of undetermined character, which, for convenience, will be termed "gummy substances." These gums may occur as intercellular masses or strands of homogeneous materials, yellowish or reddish in the unstained condition and stainable with safranine. Necrotic cavities of intercellular origin, which eventually include a zone of collapsed parenchyma, are filled with the gum, occurring either as irregular masses and strands (pl. 3, *A*, *B*) or as discrete globules. The gum may be completely amorphous or it may exhibit some striation and minute granularity. Occlusion of xylem elements often occurs, and less frequently of the phloem also. In view of the varying degrees of gum organization, from amorphous masses to sharply defined globules, the gummosis shown in plates 1, 2, *A*, and 3, *A*, *B*, may well be of essentially the same character.

A further advance in the apparent organization of secretions is exhibited by intracellular spherical bodies of the same nature as those observed in the leaves (pl. 3, *D*). In size they range from minute dots to large spheres that nearly fill the cell. They resemble the gum globules just described but have a more pronounced pelliclelike covering. Many of the bodies have protuberances, which, in fresh material, bear a superficial resemblance to budding or germination. Bodies of essentially identical structure are known to occur in leaves of various hosts infected with several species of rust. Although they are found most frequently in the nodal region, their distribution is not limited to any particular portion or tissue of the stalk. One or several may occur in a cell and may lie close to the nucleus without any evident effect on it. Rarely the nucleus partially surrounds the body, although not coming in direct contact with it, but this may be a mere physical accommodation to the presence of a foreign substance. The inclusions stain readily with safranine, iron-alum haematoxylin, and gentian violet, but are not stained by the Feulgen technique. In spite of their reaction to most nuclear stains, however, their contents are uniformly homogeneous, with no indication of a nuclear structure. Dufrenoy's description of coacervates [6] suggests these bodies, except that in the present study they have not been found in plants known to be free of chlorotic streak.

One phase of the study reported here was concerned with a search for a tangible causal organism; therefore, no intensive effort was made to demonstrate the cellular inclusions and abnormalities known to be associated with some virus diseases. Such studies are now in progress. The only abnormality to be reported at present is the presence of marked attenuations and lobes on many of the nuclei in diseased tissues. Although the nuclei of healthy tissues exhibit some small, pointed processes, the incidence of lobing, forking, and bizarre-shaped nuclei is much greater in diseased plants.

In the lower internodes of many diseased stalks, and particularly in those below the soil level, there are sporadic accumulations of clumps or strands of a gelatinous substance, which, in the fresh material, suggests organized plasmodiumlike bodies. Some of these

[6] DUFRENOY, J. VACUOLAR INCLUSIONS IN CELLS OF SUGAR CANE AFFECTED WITH CHLOROTIC STREAK. (Abstract) Phytopathology 32:3. 1942.

are spherical, some are elongate and sinuous, and some are highly irregular in outline. In size they vary from small spheres to clumps of globules that appear to fill some cells. In its more granular texture and by the presence of many small, dark, highly refractive particles, this substance differs from the amorphous masses and discrete spherical globules of gum described previously. Some of the clumps are translucent; others are dense, dark-colored, and opaque. They occur in the pith parenchyma and in vascular tissues of the stems, more abundantly in young shoots than in old stalks, but they have not been observed in the leaves; nor have they been found in plants known to be free of chlorotic streak. These bodies appear to be similar to those interpreted by Carpenter [7] as chytrids.

Numerous attempts were made to preserve these bodies by the paraffin and celloidin methods. Freehand sections were killed and examined as wet mounts in the killing fluid. The plasmodiumlike bodies were located, and the dehydrating agent was introduced under one side of the cover glass and drawn through with blotting paper. The graded dehydrating series customarily employed in the paraffin process was used. The bodies were thus under observation during the entire process of dehydration and transfer to the paraffin solvent. By selecting fields of view having distinctive features, such as the series of trachea in plate 2, *B, C,* it was possible to locate and photograph the same field at any stage of the process, even after the slide was subjected to an interval in the oven.

In these experiments, some shrinkage of the plasmodiumlike material occurred in formalin-acetic-alcohol, but the substance remained intact through prolonged storage in these fluids. It persisted during dehydration with ethyl alcohol, tertiary butyl alcohol, and dioxane, but disappeared almost completely in xylol, chloroform, and ethyl ether; and in anhydrous dioxane at 53° C. In some instances, fragments of the material persisted until paraffin infiltration was begun, but they disappeared completely during infiltration at 53°. The slow process of embedding in cold celloidin was also tried, and in some instances the substance was preserved. However, in the sections cut from this embedded material, the depositions disappeared when the stained slides were cleared in xylol.

The techniques used in this study preserve with satisfactory fidelity the plasmodia and nuclei of *Synchytrium, Plasmodiophora,* and some other Myxomycetes. It is improbable that all stages of the life history of a thalloid pathogen in chlorotic streak could have eluded the present investigators.

When fresh or preserved sections were stained with safranine and fast green, the safranine was not retained by the bodies described above; the green dye was taken up to about the same extent as by the host cytoplasm, and a metallic sheen was imparted to the dark particles within the masses. There was no evidence of nuclear structure in the masses in either freehand or fixed material. In freehand sections stained with Sudan III, the masses took on the brilliant color of the dye. This, together with its solubility in fat solvents, suggests that the material may be fatty. While this material has not been definitely identified, it is apparent from the evidence presented that it is nonliving, and it is believed to be a product of the metabolism of diseased plants rather than a causal agent.

[7] See footnote 3, p. 201.

SUMMARY

A study was made of the pathological histology of buds, leaves, and stems of sugarcane affected with chlorotic streak. Some axillary buds from diseased stalks showed no histological abnormalities; others exhibited varying degrees of necrosis of epidermis and mesophyll.

Development of the characteristic streaks on the leaves resulted in marked reductions in the number, size, and stainability of the chloroplasts in the mesophyll and chlorophyll-bearing bundle-sheath cells. When necrosis developed in these streaks, it was initiated in the mesophyll and involved the vascular bundles only after destruction of mesophyll tissue was far advanced. In diseased leaves having a "scalded" appearance, but lacking the distinctive streaks, thickening of the walls and gummosis sometimes occurred in the xylem and phloem in advance of, or simultaneously with, similar degeneration in the mesophyll.

Necrosis of parenchyma tissue of stems was found sporadically. Gummosis of the conductive elements was common, but no other abnormalities were observed in these tissues. The gumlike material may be completely amorphous; it may exhibit some striation and minute granularity; or it may be aggregated into spherical or teardrop-shaped globules.

Spherical intracellular bodies that stained deeply with safranine and iron-alum haematoxylin were found in stems and leaves of diseased plants. Although some of these had a pelliclelike covering resembling a wall, their contents were homogeneous, with no indication of nuclear structure.

Accumulations of a gelatinous substance, frequently resembling organized bodies, were observed in the stems of some diseased plants. The material was soluble in ether, xylol, dioxane, and chloroform, but not in ethyl alcohol, and stained brilliantly with Sudan III, indicating a fatty chemical character.

The various types of gummosis and the bodies exhibiting some degree of structural organization are believed to be products of the metabolism of diseased plants, rather than causal agents.

THE Ms$_2$ A V$_{10}$ LINKAGE GROUP IN SORGHUM [1]

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INTRODUCTION

The male-sterile character in sorghum (*Sorghum vulgare* Pers.) offers the most feasible method yet devised of producing first-generation hybrid seed in large quantities and of thus utilizing the hybrid vigor found in certain sorghum crosses for obtaining high yields. Since male-sterile plants obviously are not self-propagating and the character must be maintained in a heterozygous condition, all pollen-producing segregates must be removed from the seed-parent variety growing in a crossing block. Such plants must be destroyed before any pollen is shed, and the earlier they can be removed from competition with the ultimate seed-producing plants the greater will be the acre yield of hybrid seed. Any easily recognized plant character that is closely associated with male sterility by genetic linkage would permit the removal of most of the pollen-producing plants before the pollination stage is reached. Data are presented to show the association of factor pairs for awnless and awned lemmas and for green and virescent-yellow plants with the male-sterile factor pair, *Ms$_2$ms$_2$*.

REVIEW OF LITERATURE

Male sterility in sorghum was reported by Rangaswami Ayyangar and Ponnaiya (*1*)[2] and by Stephens (*15*), in 1937. In each case anthers were devoid of pollen but stigmas were receptive; monogenic inheritance was observed with male sterility recessive; and the symbols *Msms* were used for the factor pair involved. Karper and his associates (*9*) reported two additional male steriles and designated them *ms$_3$* and *ms$_4$*, but they gave no data regarding inheritance.

In the cross Dwarf milo × feterita, Vinall and Cron (*18*) reported the F$_1$ unawned and a segregation of 3 awnless to 1 awned in the F$_2$. Sieglinger et al. (*14*) described milo as strong-awned, feterita as tip-awned, and the F$_1$ as weak-awned and found that the segregation was 1 strong-awned, 2 weak-awned, and 1 tip-awned in the F$_2$ of crosses between strong-awned and tip-awned varieties. They considered strong-awned partly dominant to tip-awned. In crosses involving awnless and either tip-awned or strong-awned they found the awnless condition to be almost completely dominant in the F$_1$. They reported 3 : 1 segregations in the F$_2$ with awnless dominant.

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To explain their results they suggested multiple allelomorphs and used the symbols AA (awnless), aa (strong-awned), and $a^t a^t$ (tip-awned) for the factors involved. They also recognized homozygous weak-awned strains but did not report on the inheritance of this type of awn development. Ramanathan (*12*) reported short awns dominant to long awns, and Karper (*6*) and Sieglinger (*13*) reported awnless dominant to awned.

Rangaswami Ayyangar and Reddy (*2*) reported a type of stigma on which the brushy or feathery branches occupied only the lower third of the axis while the upper two-thirds was devoid of branches. They found basal-feathered stigmas to be recessive to normal, or full-feathered, with monogenic inheritance (*3*). They also reported that in most groups of sorghum leaf tips are hairy but that in a few groups they are usually glabrous (*4*). Hairy leaf tip was a monogenic dominant to glabrous leaf tip. In crosses where both pairs of characters were brought together in the coupling phase, these workers found linkage with 25 percent crossing over. The factors for each of the characters were also linked with those for awns. In the F_2 and F_3 populations crosses with leaf tip hairy vs. glabrous and awns absent vs. present gave 43 percent crossing over in the repulsion phase, and crosses with awns absent vs. present and stigmas full-feathered vs. basal-feathered gave 18 percent crossing over. These data established the existence of a linkage group that includes the factor for the presence of awns, with percentages of crossing over between successive factor pairs in the following order: Awns (18) stigma feathering (25) leaf tip hairiness. Each pair of this group was reported to be independent of factors for plant color (Pp), wholeness of grain color (Ww), and hairiness of nodal band. Sieglinger (*13*) reported awns independent of seed-color factors in the F_2 of a Blackhull kafir × darso cross, and Stephens and Quinby found no association of awns with members of the $Q\ B\ Gs$ (*16*) and $D\ Rs\ P$ (*17*) linkage groups.

A number of chlorophyll-deficient types, ranging from those with weak zygotes, some of which fail to emerge, and from albinos lethal as soon as endosperm food reserves are exhausted, to pale greens that can hardly be separated from normal siblings, have been reported in sorghum. Karper and Conner (*8*) designated two virescents found in Blackhull kafir v_1 and v_2 and showed data indicating monogenic inheritance in each case. A third chlorophyll deficiency, described in the text as pale yellow but in the summary as virescent, was assumed to be a simple recessive. This virescent was later (*6*) designated v_3 and reported independent of seedling red stem (Rs), w_1 and w_2 (albinos), and y_2 (lethal-yellow seedling). In 1935, Karper (*7*) reported six virescents obtained from X-rayed seed, and these are the six for which Martin (*11*) used the symbols v_3 to v_8, inclusive, with the data credited to Quinby and Karper. The symbols for the X-ray virescents should have been reported to Martin as v_4 to v_9, inclusive. It has not been determined definitely that no duplications occur within this series.

DESCRIPTION OF CHARACTERS

MALE STERILITY

In typical expression, anthers of male-sterile ($ms_2 ms_2$) plants are one-half or less the size of normal anthers and do not contain pollen (fig. 1, *A* and *B*). Though readily located in normal siblings, no pollen mother cells could be found in male-sterile plants, indicating that
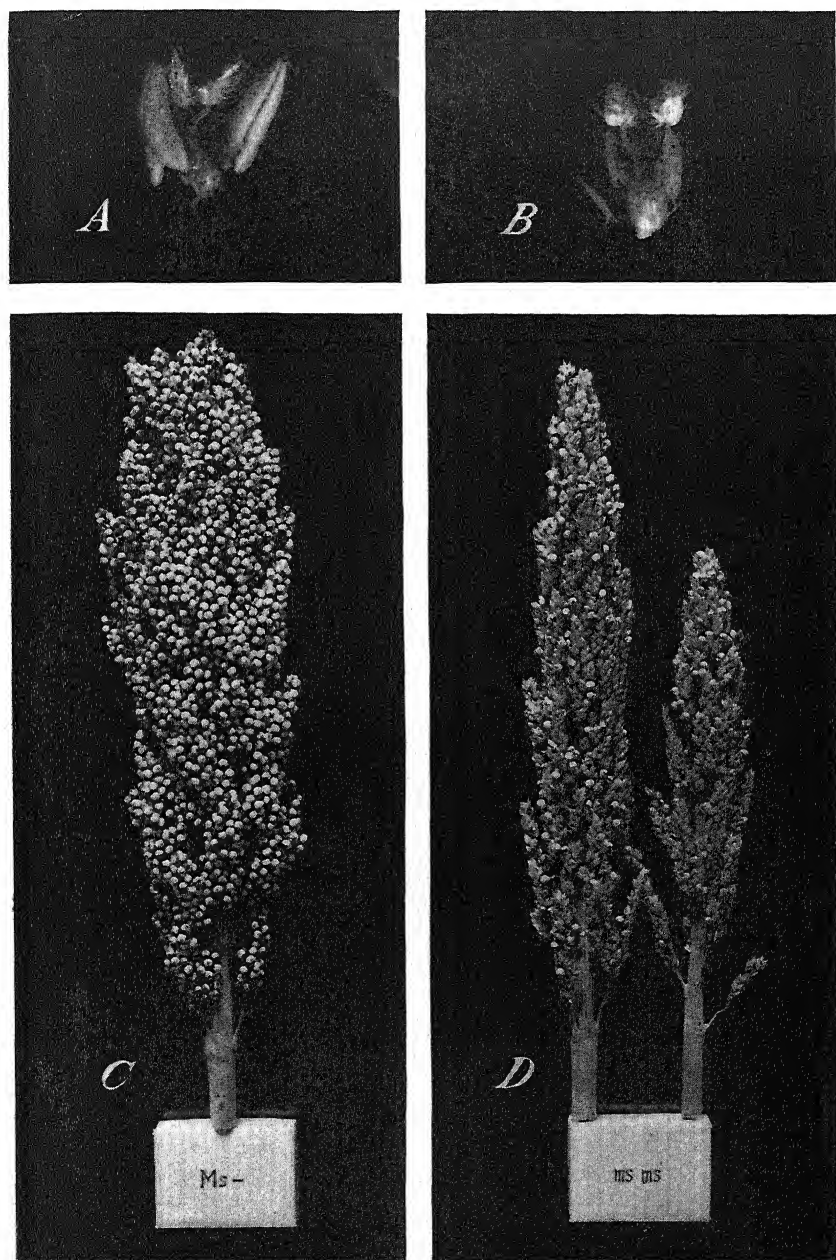
FIGURE 1.—Flowers of normal (A) and male-sterile (B) Texas Blackhull kafir, showing relative size of anthers.   Panicles of normal (C) and male-sterile (D) Texas Blackhull kafir.   Note pointed apex of D.

action of the gene takes place before the formation of pollen mother cells. However, in occasional populations the anthers do reach a more advanced stage, and in at least three of the populations grown, scattered normal flowers have been observed on male-sterile panicles. In the Texas Blackhull kafir stock, male-sterile panicles are distinctly more pointed than normal panicles, so that one can make fairly accurate separations of phenotypes by observing panicle shape as heads are exserted from the upper sheaths (fig. 1, *C* and *D*). Ordinarily the panicle of Blackhull kafir grown under favorable conditions has a rachis 60 to 80 percent of the total panicle length, terminated by a cluster of branches (*19*). The pointed appearance of the male-sterile panicle results from a longer rachis and shorter rachis branches toward the tip than is common in the normal panicle. This distinction is less apparent in many stocks with panicles of other shapes. Otherwise the phenotypes are similar in appearance, except for more side branching on male-sterile plants because of incomplete fruiting in the main head (fig. 2). Wind and insect pollinations result in producing seed in unbagged male-sterile panicles, but the amount produced varies considerably.

AWNS

In recording the results of this investigation, long awns were considered recessive. Most of the populations segregated in the ratio of 3 absent (not showing above apices of glumes) to 1 long. No attempt was made to determine the character of awns not exposed. In a few crosses, where awns were tip, short, and long, the first two classes were recorded together because they were often hard to separate, whereas phenotypes with long awns were easily recognized.

VIRESCENCE

Since nine previously reported virescents in sorghum have been designated v_1 to v_8, inclusive, and correction for the duplication extends the series to v_9, the symbols $V_{10}v_{10}$ are used for the factor pair responsible for the green and virescent phenotypes reported here. In stocks in which the character is well expressed, the virescent seedlings emerge a rich yellow to a greenish yellow, in sharp contrast to normal green seedlings, and successive leaves emerge yellow. At a later stage, which varies considerably with the stock and growing conditions but usually is the stage when three or four leaves are out, the tip of the lower leaf begins to turn green and the green color gradually extends to successive leaves up the stalk and to the bases of the blades and the sheaths. Often the panicle emerges greenish yellow, but shortly after emergence the plant becomes entirely green and cannot be distinguished from normal siblings unless new growth in the form of tillers or branches is present.

Expression of virescence is quite variable. In some homozygous virescent stocks a fairly high proportion of the seedlings die at an early stage, whereas in others many of the seedlings are so green that they cannot be satisfactorily separated from normal green seedlings. In some stocks various degrees of green striping occur. No segregations have yet been obtained to give a satisfactory explanation of these variations on the basis of definite modifiers that could be identified, and so far no emerging panicle has been observed in which the striping could be traced into the panicle distinctly enough to divide the panicle into sectors. No data are used in this paper from segregat-
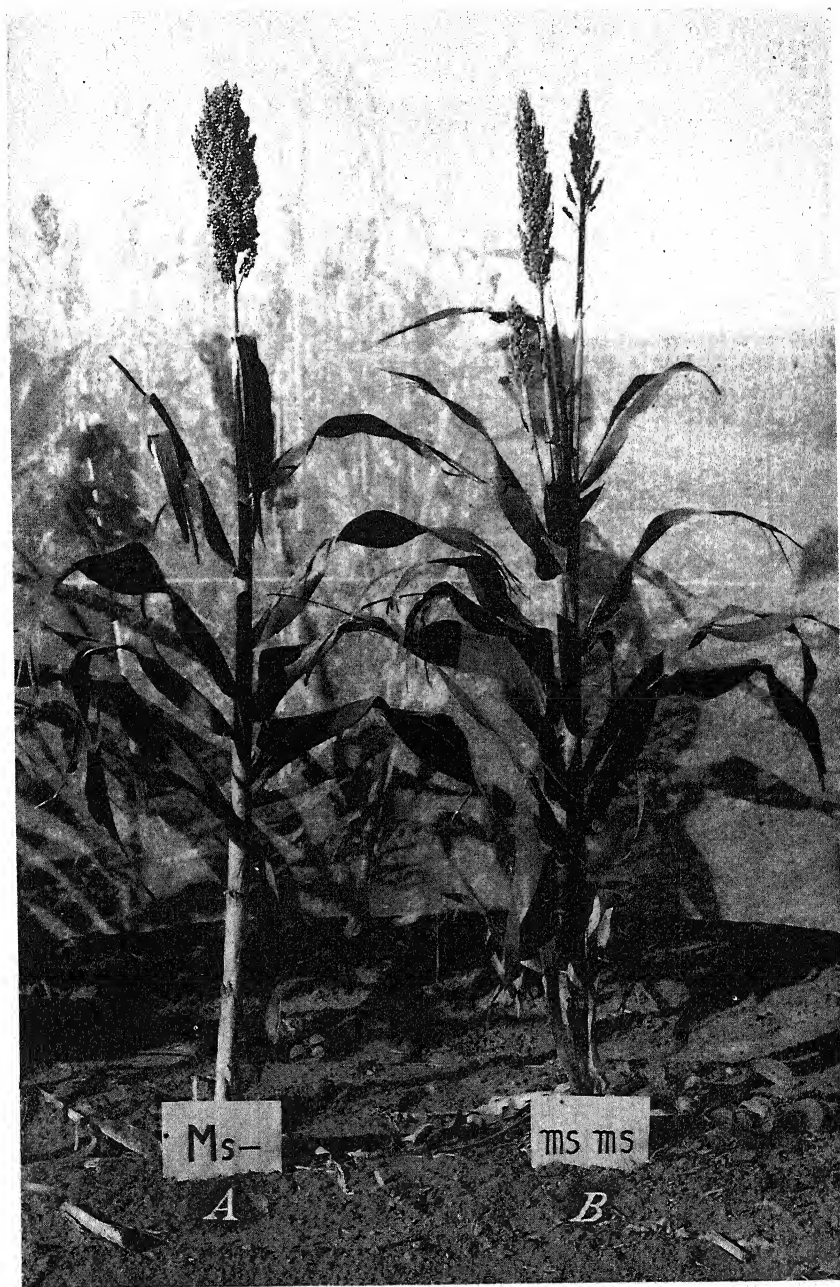
FIGURE 2.—Texas Blackhull kafir, showing relative side branching on normal (*A*) and male-sterile (*B*) plants.

ing populations in which there was any question of the identity of the virescent seedlings. All virescents were staked in the late-seedling stage.

Factors for awns and male sterility have been found independent of V_2v_2, but none of the previously reported virescents has been tested directly with v_{10}. In the stocks observed, v_{10} has appeared to differ in expression from v_1 to v_9, inclusive, and is not likely to prove to be a duplicate of any of these.

CROSSES BETWEEN MALE-STERILE 1 AND 2

Through the courtesy of G. N. Rangaswami Ayyangar, seed of a stock carrying factors for male sterility in durra found at Coimbatore, India, was received at Chillicothe, Tex., in the spring of 1939. Because of short-day requirements, no plants headed in that year and only 3 seeds remained in the spring of 1940. These seeds produced 3 plants which were grown under a 9-hour photoperiod. All 3 plants were normal, and pollen from each was dusted on the stigmas of male-sterile plants of Texas Blackhull kafir. From these crosses, 15 F_1 plants, grown in a greenhouse at Washington, D. C., by J. H. Martin, produced normal anthers and set seed under bagged heads, and 28 F_1 plants, grown in the field, produced normal anthers and pollen but did not mature before frost. In 1941, small populations from seed of each of the 3 Coimbatore durra plants and from the 15 F_1 plants were grown under a 10-hour photoperiod. Each of the Coimbatore populations contained male-sterile plants, indicating that the parents were heterozygous for male sterility. All the F_2 populations except 1 contained male-sterile plants, and these populations can be classed roughly in 2 groups, which segregated in the ratios of 9 : 7 and 3 : 1 for male sterility (table 1). On the basis of independent factors for the 2 male-steriles, half of the populations should fall in each group. These F_2 populations are too small to show definitely that a given one belongs in the 9 : 7 or 3 : 1 group, but the evidence is sufficient to allow the conclusion that different genes are responsible for the 2 male-steriles. In this paper, factors for the durra normal allelomorph and male-sterile are indicated by the symbols Ms_1ms_1 and for the kafir normal allelomorph and male-sterile, by Ms_2ms_2.

TABLE 1.—*Independent segregation in the F_2 of durra male-sterile (ms_1) and kafir male-sterile (ms_2) in the cross kafir male-sterile ($Ms_1Ms_1ms_2ms_2$) $\times$ heterozygous durra male-sterile ($Ms_1ms_1Ms_2Ms_2$)*

Duplicate-factor segregation (9 : 7 ratio)				Kafir male-sterile segregation (3 : 1 ratio)			
	Plants of indicated phenotype				Plants of indicated phenotype		
Plot No.	Normal	Male-sterile	Total	Plot No.	Normal	Male-sterile	Total
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
4	12	15	27	5	15	4	19
6	18	7	25	8	9	2	11
7	8	19	27	10	7	2	9
9	9	7	16	13	2	1	3
11	16	6	22	15	11	4	15
12	2	8	10	16	13	4	17
14	18	13	31	17	12	3	15
				18	8	0	8
Total	83	75	158				
Expected	89	69		Total	77	20	97
				Expected	73	24	

SINGLE-FACTOR INHERITANCE

Male-sterile segregates in the groups of populations shown in table 2 were consistently and, except for a few small populations, significantly short of expected numbers. This deficiency of male-sterile phenotypes has been rather general, but the cause has not been determined.

Most segregating populations had a shortage of the expected number of virescents. The original deficiency varied in different stocks, and survival from seedling stage to maturity was nearly always at least a little greater among normal siblings than among the virescents.

Segregation of awns deviated significantly from expected ratios in several groups. Some of these deviations may have resulted from the association of awns with male sterility and virescence.

LINKAGE

The linkage of factors for male sterility and awns, awns and virescent yellow, and male sterility and virescent yellow is shown in table 2. These crosses were among many made primarily to find characters associated with male sterility or to develop lines with increased seed-setting capacity on male-sterile plants, which accounts for the large number of different but related populations grown over a period of several years. All the data are from two-factor segregations. Several F_2 and backcross populations with the three-factor pairs segregating in the coupling phase were grown in 1941. A heavy infestation of sorghum midge (*Contarinia sorghicola* (Coq.)) and the occurrence of late-autumn rains, which delayed tabulations until early December, made it impossible to classify all male-sterile and normal phenotypes with certainty. Data from part of the populations were tabulated for these characters, but they appeared inconsistent with those previously accumulated and were discarded. Data from these populations were included, however, for determining linkage between awns and virescent yellow.

The cross-over percentages (table 2) appear rather variable though they are perhaps not more inconsistent than might be expected, considering the nature of the material from which the data were collected.

Weighted averages of all linkages (table 3) indicate the order of genes with approximate cross-over percentages to be as follows: ms_2 (10.9) a (9.1) v_{10}, or total cross-overs between ms_2 and v_{10}, about 20 percent. The weighted average of cross-over percentages where only the factor pairs Ms_2ms_2 and $V_{10}v_{10}$ were segregating is 19.3; with an expected coincidence of about 1 percent, this value is close to the sum of the other two values.

The characters stigma feathering and leaf-tip hairiness reported by Rangaswami Ayyangar and Reddy (*4*) as associated with awns were not investigated. The basal-feathered type of stigma has not been observed in any of the Chillicothe stocks, and variations in leaf-tip hairiness could not be separated into definite phenotypes. Consequently, whether factors for these characters are on the ms_2 or v_{10} side of those for awns has not been determined.

TABLE 2.—*Linkage of male sterility (Ms₂ms₂), awns (Aa), and virescent yellow (V₁₀v₁₀) in F₂ and backcross populations*

Year	Genes A B	Linkage phase	Populations	AB	Ab	aB	ab	Total plants	Cross-over [1]	Standard error	aa Percent	aa χ²	aa P	bb Percent	bb χ²	bb P
			Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*	*Percent*						
1937	Ms₂A	RS	3	81	47	38	1	167	14.5	7.5	23.35	0.286	>0.05	28.74	1.145	>0.05
1938	do	do	3	308	153	102	0	563	[2]10.0	4.2	18.12	14.392	<.02	27.18	1.363	<.05
1939	do	do	8	792	329	265	2	1,388	9.5	2.7	18.24	24.592	<.02	23.85	.984	<.05
1940	do	do	6	433	189	145	3	770	15.0	3.5	19.22	13.716	<.02	24.94	.002	<.05
Total or weighted average			20	1,614	718	550	6	2,888	11.0	1.8	19.25	50.888	<.02	25.07	.007	>.05
1940	Ms₂A	CS	15	1,080	93	56	253	1,482	11.0	.9	20.85	13.611	<.02	23.35	2.160	<.05
1941	do	CB	1	53	7	2	29	91	9.9	3.1	34.07	9.242	<.02	39.56	3.967	<.05
1940	AV₁₀	RS	2	135	54	73	0	262	[2]13.0	6.0	27.86	1.145	>.05	20.61	2.692	<.05
1938	AV₁₀	CS	2	135	7	15	38	195	11.0	2.4	27.18	.436	>.05	23.08	.436	<.05
1940	do	do	3	112	5	11	30	158	10.0	2.5	25.95	.076	>.05	22.15	.684	<.05
1941 [3]	do	do	21	2,144	116	122	578	2,960	8.5	.5	23.65	2.883	>.05	23.45	3.813	<.05
Total or weighted average			26	2,391	128	148	646	3,313	9.0	.5	23.97	1.861	>.05	23.36	4.695	<.05
1941 [3]	AV₁₀	CR	2	178	18	21	174	391	10.0	1.5	49.87	.003	>.05	49.10	.125	<.05
1941 [3]	do	do	4	232	28	12	180	452	8.8	1.3	42.48	10.230	<.02	46.02	2.867	<.05
Total or weighted average			6	410	46	33	354	843	9.4	1.0	45.91	5.648	<.02	47.45	2.193	>.05
1937	Ms₂V₁₀	RS	1	105	17	38	0	160	[2]26.0	7.3	23.75	.133	>.05	10.63	17.633	<.02
1940	do	do	1	167	75	64	3	309	21.5	5.4	21.68	1.730	>.05	25.24	.017	<.05
Total or weighted average			2	272	92	102	3	469	20.0	4.4	22.39	1.640	>.05	20.26	5.512	<.02
1940	Ms₂V₁₀	CS	15	897	123	95	190	1,305	19.0	1.2	21.84	6.874	<.02	23.98	.691	<.05
1941	do	CB	8	301	69	57	211	638	19.7	1.6	42.01	16.307	<.02	43.89	9.536	<.02

[1] In F₁ populations; estimated to nearest half percent by use of Immer's tables (6).
[2] Assuming one phenotype in double-recessive class.
[3] 3-point tests, but data used only for region Aa to V₁₀v₁₀.

TABLE 3.—*Summary of linkage data for male sterility (Ms₂ms₂), awns (Aa), and virescent yellow (V₁₀v₁₀)*

Linkage phase	Genes								
	Ms_2A			AV_{10}			Ms_2V_{10}		
	Popula-tions	Total plants	Cross-over	Popula-tions	Total plants	Cross-over	Popula-tions	Total plants	Cross-over
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
R.S.	20	2,888	11.0±1.8	2	262	13.0±6.0	2	469	20.0±4.4
C.S.	15	1,482	11.0±0.9	26	3,313	9.0±0.5	15	1,305	19.0±1.2
C.B.	1	91	9.9±3.1	6	843	9.4±1.0	8	638	19.7±1.6
Weighted [1] average			10.9			9.1			19.3

[1] Inversely as the squares of standard errors (*10, p. 325*).

SUMMARY

This paper reports a linkage group in sorghum of three pairs of genes. The phenotypes are normal and male-sterile flowers, awnless and awned lemmas, and green and virescent-yellow plants. The factor pairs are designated Ms_2ms_2, Aa, and $V_{10}v_{10}$, respectively, the symbols for awns having been used previously in other papers. The factors Ms_2 and V_{10} show complete dominance over their respective allelomorphs in the F_1 and the factor A almost complete dominance. Single-factor segregation is indicated in F_2 and backcross populations, although in most of the populations recessive classes were short of the expected numbers. The indicated order of genes with cross-over percentages is ms_2 (10.9) a (9.1) v_{10}.

Since the factors for the easily observed characters, awns and virescent plants, are linked with the factor for male sterility, a guide is provided for the removal before pollination begins of a large proportion of the normal plants from a population segregating for male sterility.

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GROWTH CURVES OF AZOTOBACTER AT DIFFERENT pH LEVELS [1]

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INTRODUCTION

Adequate data have been accumulated during the past three decades to show that the H^+ concentration of the medium in or upon which *Azotobacter* is grown exercises a marked influence upon certain of its physiological activities. Students of this organism have placed special emphasis upon the effect of the H^+ concentration 077: (1) Its distribution in soils; (2) its ability to bring about the fixation of uncombined atmospheric nitrogen; (3) its respiration; and (4) its growth.

The available data relative to be influence of the H^+ concentration upon distribution and nitrogen fixation point rather conclusively to a limiting H^+ concentration, expressed as pH, in the vicinity of 6.0 for most strains of *Azotobacter*. For nitrogen fixation Burk and his colleagues (*3, 4*) [2] recorded the specific limiting pH value of 5.97, and suggested that at lower pH values the nitrogen-fixing enzyme is inactivated.

In view of the variability recorded for other characteristics of *Azotobacter*, it is questionable whether one should expect a single specific limiting H^+ concentration for the various physiological activities of this genus, or even a constant limiting H^+ concentration applicable to all strains for any single function. In fact, Starkey (*11*) has shown that for at least one strain or species the limiting H^+ concentration for activity in general approximates pH 3.0 instead of pH 6.0.

According to Burk and his colleagues (*2*), who have investigated the influence of the H^+ concentration upon respiration of *Azotobacter* extensively, limited temporary respiration may take place at pH levels of 5.0 or less. However, a rapid decrease in the respiration rate was recorded as the pH fell below 6.0, accompanied by a gradual inactivation of the enzyme system involved. The lower the pH level the less active and the less stable the enzyme system became.

The data relative to growth are less conclusive. Burk and his associates (*4*) observed no growth of *Azotobacter* in a medium free of combined nitrogen when the pH fell below 5.97. On the other hand, measurable growth was noted at pH values of 4.0 to 4.5 when fixed nitrogen was included in the medium. This would indicate that the processes involved in growth continued active at H^+ concentrations inhibitory to the metabolism of elementary nitrogen. This fact alone is not particularly surprising. If the nitrogen-fixing enzyme system is inactivated at a pH value of 5.97, no growth could be expected in a medium free of combined nitrogen at pH values below that point, since available nitrogen would become the limiting factor in growth.

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However, if "Respiration gradually ceases or comes to an insignificant value at pH 6.0 as the result of an integrated combination of reversible and irreversible time factors" (*2, p. 438*), it is difficult to see how growth could continue below pH 5.97 even in the presence of combined nitrogen.

Furthermore, if under natural soil conditions, *Azotobacter* metabolizes only fixed nitrogen (*1*), as it apparently does in the laboratory where adequate combined nitrogen is available, it is also difficult to understand why it is not found in abundance in fertile soils with pH values below 6.0. Such soils furnish adequate quantities of combined nitrogen for the abundant growth of higher plants and saprophytic micro-organisms in general. During the growing season, such soils contain appreciable quantities of nitrate and other forms of nitrogen known to be available to *Azotobacter*, and there is no evident reason why it should not compete successfully with other soil saprophytes and higher plants for this nitrogen. The mere fact that *Azotobacter* is seldom found naturally (*9*) in such soils and disappears from them when introduced artificially (*8, 10*) is a priori evidence that growth does not take place in them. Such discrepancies suggested the desirability of further investigations relative to the influence of H^+ concentration upon the growth of *Azotobacter*.

EXPERIMENTAL PROCEDURE

Numerous preliminary experiments involving over 40 strains of *Azotobacter* were carried out on washed mannitol agar slants adjusted to different pH levels. Observations in parallel of growth on a nitrogen-free medium and in the presence of fixed nitrogen (KNO_3) were recorded by this procedure. Only freshly prepared slants were used, and uniform inoculation was obtained by employing one drop of a heavy suspension of young cells as the inoculum for each culture in any single experiment. This procedure obviously did not lend itself to accurate quantitative measurements of growth. Visual records of growth taken after 48, 96, and 168 hours gave valuable qualitative information, particularly as to the range in pH value over which subsequent studies of different strains should be conducted.

Most of the data here recorded were obtained by growing the various strains in vigorously aerated liquid media adjusted to different pH values. After suitable incubation periods quantitative measurements of turbidity, volume of cells, pH level, microscopic count of cells, and in some instances, energy material consumed, served as criteria of growth.

The medium used was the usual salts medium composed of 3.0 gm. of a mixture of KH_2PO_4 and K_2HPO_4 (the ratio depending upon the approximate pH value to which it was desired to buffer the basic medium); 0.2 gm. NaCl; 0.2 gm. $M_9SO_47H_2O$; 0.02 gm. $CaCl_2$; 0.02 gm. $CaCO_3$; 3 drops of a 10 percent soloution of $FeCl_3$; 3.0 p.p.m. MoO_3; 1,000 ml. distilled water; and 20.0 gm. mannitol or other energy material. Fixed nitrogen was supplied in the form of ammonium sulphate in a concentration of 100 p.p.m., unless otherwise stated. An ammonium salt was employed because any appreciable utilization of the ammonium ion would increase the H^+ concentration of the medium, and thus tend to inhibit rather than to stimulate growth. Two hundred milliliter quantities of the medium were sterilized in 300 milliliter pyrex Erlenmeyer flasks. Aerating tubes

inserted in nonabsorbent cotton stoppers wrapped in gauze were sterilized separately and inserted after inoculation.

The inoculum was grown on mannitol agar contained in Blake bottles usually for 18 to 24 hours or longer, depending upon the rate of growth, and suspended in sterile culture medium. Where gum formation was abundant it was found advantageous to shake the suspension of cells thoroughly in a flask containing a few sterile glass beads. Carefully measured quantities of such a suspension of cells were added to each flask in a given experiment to insure uniform inoculation. No effort was made to obtain a constant concentration of cells in the inocula employed in different experiments; hence the different experiments cannot be compared directly insofar as the initial number of cells is concerned. However, a glance at the various charts subsequently presented will show that cessation of growth was in no way related to number of cells.

Minor variations in the pH level of the culture medium were obtained by adding the necessary quantity of NaOH or HCl. Because of the difficulty in maintaining seals where pressure is employed and frequent sampling is necessary, each flask was attached separately to the compressed air system. This led to some variation in the rate of air flow through the different cultures in a given experiment, but observation has shown that where vigorous bubbling of air through cultures is maintained, variation in growth between replicated flasks is not very great. Incubation was at 28° to 32° C. Aeration for 30 minutes preceded sampling for the initial analysis, thus insuring a homogenous distribution of cells.

A Petroff-Hausser counting chamber was employed for determining the number of cells. If the concentration of cells was high, a preliminary dilution was made. Difficulty was encountered in attaining the high degree of accuracy in counting cells that has been reported for other cultures (5). It was therefore desirable to make a sufficient number of replicate counts to permit statistical treatment of the data. Hence, in most instances reported, the entire ruled area of five separate mounts was counted. It was impossible to complete all the other quantitative measurements and make a larger number of counts without consuming so much time that significant changes might have taken place before all analyses could be completed. The data have been subjected to Fisher's (7) t test for significant differences in the number of cells at the different analyses. Because of the difficulty of indicating such calculated values graphically it has been arbitrarily assumed that when the calculated t value was less than the t value in Fisher's table at the 5 percent level the increase was not significant. This has been indicated on the accompanying graphs by an arrow pointing to the last analysis beyond which no such increase was recorded.

Where active reproduction of *Azotobacter* in aerated liquid cultures is taking place, cells may be observed in all stages of division, but little tendency to adhere together after division is noted. Since the point at which a dividing cell should be regarded as two individual cells is at best arbitrary, all dividing cells were counted as single units. In spite of continuous agitation due to aeration and vigorous shaking immediately before sampling, composite counts obtained by this procedure were occasionally obviously out of line. As yet no practicable means of eliminating such errors has been devised.

Turbidity was measured with the aid of a Gates suspensiometer and readings have been recorded in the reciprocal of depth in centimeters of suspension necessary to obscure the wire loop.

The volume of cells was determined by centrifuging a measured volume of the culture in a Hopkins vaccine tube and reading the volume directly. Many of the data obtained by this method were obviously unsatisfactory. Cultures occasionally flocculated and the degree of packing appeared to be influenced also by the presence of gum which varied widely among the different strains and possibly with other factors such as age.
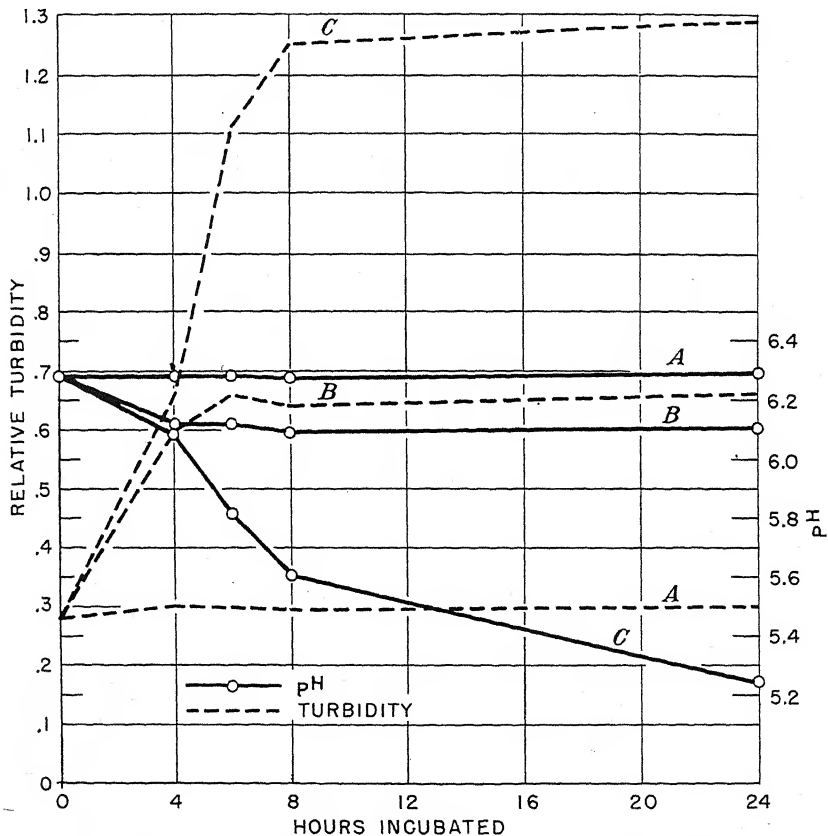
FIGURE 1.—Influence of H_9Cl_2 upon pH and turbidity curves, *Azotobacter* strain 32. *A*, H_9Cl_2 added initially; *B*, H_9Cl_2 added after 4 hours' incubation; *C*, no H_9Cl_2 added. Note constancy of pH and turbidity values in the presence of H_9Cl_2; i. e., in the absence of bacterial activity.

Hydrogen-ion concentration was measured at each analysis with the aid of a glass electrode and, as indicated in figure 1, replicated determinations made under conditions where no changes would be expected checked within a few hundredths of a pH unit even though 24 or more hours intervened between readings.

Following the final analysis, and frequently at each analysis, every culture was checked for purity by streaking on plates of both nutrient and mannitol agar and, after adequate incubation, examining the

growth carefully under the 16- and 4-mm. objectives for indications of contamination. All data obtained from cultures showing indications of contamination were discarded. Contaminants were easily detectable only when cultured on nutrient agar.

EXPERIMENTAL DATA

GROWTH ON SOLID MEDIA

In table 1 are recorded the data from two experiments in which the growth of 37 strains of *Azotobacter chroococcum* Beij. was observed on washed mannitol agar adjusted to different pH levels, one series of cultures being grown on nitrogen-free agar, the other on the same medium containing fixed nitrogen in the form of KNO_3. These two experiments were selected from among many only because they gave a better distribution of initial pH values. Growth was recorded after 2, 4, and 7 days' incubation, but the inclusion of data for the two shorter periods would add little except to indicate that frequently growth started somewhat sooner in the presence of fixed nitrogen and that near the limiting pH growth was extremely slow with all strains. In view of the ready availability of nitrate nitrogen to so many higher plants, fungi, and bacteria and the relative inertness of elementary nitrogen, the more rapid growth in the presence of the fixed nitrogen is not surprising.

These data definitely indicate that there is no significant difference in the critical H^+ concentration for the growth of *Azotobacter* in the presence or absence of fixed nitrogen, at least insofar as these 37 strains, isolated from a wide variety of soils, represent the group. Without exception, every strain grew at a pH value of 6.0 while no strain grew at a pH value of 5.39 or 5.44. An analysis of growth records at all pH values lying between 5.44 and 6.00 reveals 89 failures to grow in the absence of fixed nitrogen and 84 failures to grow in the nitrogen-containing medium. Also, there were 4 cultures for which growth was recorded at a slightly lower pH level in the nitrogen-free medium and 9 cultures in which the reverse was true.

TABLE 1.—*Growth of Azotobacter chroococcum on nitrogen-free mannitol agar and on mannitol agar containing KNO₃, at different initial pH levels*

Culture No.	Growth of Azotobacter on nitrogen-free agar with initial pH of—									Growth of Azotobacter on agar containing KNO₃ with initial pH of—								
	[1] 5.44	[1] 5.58	[1] 5.71	[2] 5.83	[2] 5.92	[2] 6.00	[2] 6.17	[1] 6.17		[1] 5.39	[1] 5.60	[1] 5.73	[2] 5.83	[2] 5.92	[2] 6.00	[2] 6.17	[1] 6.19	

[1] Experiment conducted Nov. 10, 1939.

[2] Experiment conducted Dec. 2, 1939.

Culture No. column entries: 28, 61, 60, A, O₂, 17, F2, 70, 22, 37b, 54, 11, 44, 12, 10, 21, 37, J4-b, 14b, 121, 50, 25, 6a, O2-b, V3, P1, K, 32, 24, N1, 33, 14, 13, 118, 3, 67, 34.

These data indicate some variation in the limiting H^+ concentration for growth among the different strains, the maximum variation approximating 0.5 pH unit.   In the presence of fixed nitrogen, for example, 6 strains ceased growth between pH 6.0 and 5.92; 14 between pH 5.92 and 5.83; 8 between pH 5.83 and 5.73; while 9 strains exhibited more tolerance toward hydrogen ions but ceased growth altogether before pH 5.44 was reached.   If the 48-hour records had been included it would be evident that growth of a dozen or more strains was seriously retarded at a pH value as high as 6.17, either a zero or $+$ representing growth as compared with the normal $++++$ for more favorable pH values.

In view of subsequent observations relative to changes in the pH value of cultures in which no growth, ie., no increase in cell numbers, took place, it is probable that some instances of growth are recorded in table 1 at pH levels where growth actually would not take place. The utilization of the NO_3 ion results in a marked elevation in pH (fig. 5, B_5).   If the initial H^+ concentration happened to be only slightly above the critical point it is conceivable that the cells originally introduced as the inoculum might lower the H^+ concentration sufficiently to make some growth possible.   If such is the case each successive increment of growth would render the conditions more favorable.   Some evidence in support of this view has been obtained. The method of inoculation already described resulted in a massing of cells at the butt of the slant.   In nearly all instances where limited growth $(+)$ was recorded, it took place very slowly and was confined to the butt.   In some instances where growth was questionable after 96 hours' incubation, a restreaking of cells from the butt of the slant resulted in good growth during the following 72 hours.   This would indicate that some radical alteration had taken place either in the medium or in the organisms since the original inoculation, possibly as a result of a change in the reaction of the medium or a rather rapid adaptation of the organisms to the more acid condition.   Such an adaptation does not appear to take place in soils either under natural or experimental field conditions.   Neither repeated inoculation nor the constant maintenance of *Azotobacter* in adjacent soil over a period of 20 years has resulted in the establishment of strains of *Azotobacter* in soils with pH values only slightly below 6.0.

GROWTH IN LIQUID MEDIA

Because of the much more rapid growth obtainable in aerated liquid media and the possibility of applying quantitative analytical methods, it was hoped that a more accurate determination of the end point of growth could be made by the use of such methods.   The general procedure followed was to start a given experiment early and make as many quantitative measurements of growth during the day and evening as possible, and to follow these with one anaylsis the next morning.   Data obtained by this procedure are amenable to graphic presentation and such a method has been followed.   An effort has been made to select from the mass of data accumulated during the past few years representative experiments to illustrate the major points brought out in these studies.   These data are presented in figures 1 to 11.   Each graph could be replicated a number of times if space permitted.

The primary objective was to determine the relationship, if any, between the H^+ concentration of the medium and growth of *Azoto-*

bacter. Since several criteria of growth were used and these were sometimes found to measure separate and not necessarily related factors, it will be helpful to indicate more precisely just what factors each criterion did evaluate.

The term "growth" as employed in bacteriologic literature implies, unless otherwise defined, an increase in the number of individual cells. If this concept of growth is adhered to, then a cell count should be the most, and possibly the only accurate, measure of growth. Because of certain inherent difficulties encountered in determining accurately the number of bacterial cells, other methods of estimating growth have been introduced. It has been assumed that these methods more or less accurately indicate the number of cells; hence may be employed in measuring growth.
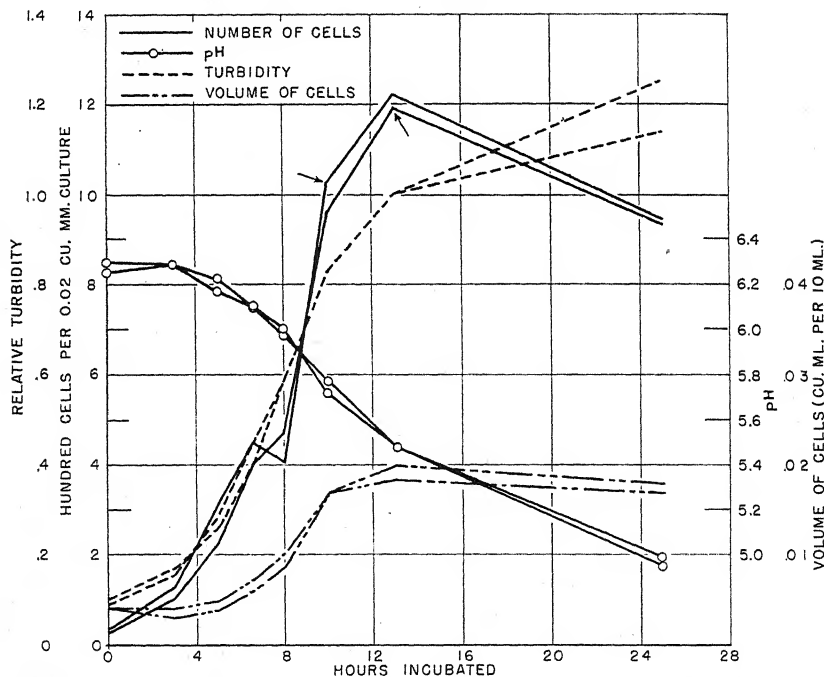
FIGURE 2.—Number of cells, pH value, turbidity, and volume of cells recorded from duplicate cultures of *Azotobacter* strain 32. Arrows indicate points beyond which gains were not significant at the 5-per cent level.

Among the indirect means employed to measure growth, turbidimetric methods rank first. The use of this procedure is predicated upon the assumption that there is a direct relation between turbidity and the number of particles (bacterial cells) in suspension, an assumption that is open to question. Turbidity results from the so called "scattering" (reflection) of light rays by the particles in suspension. Since size and composition as well as the number of suspended particles influence "scattering" phenomena, a direct relationship between turbidity and the number of suspended particles could be expected only when the particles are constant in size and composition.

As long as rapid cell division is taking place in an aerated culture of *Azotobacter*, the general appearance of the cells remains quite

similar. A glance at figures 2, 6, 8, and 9, will show that during this phase of growth the curves for turbidity and number of cells are similar. The smoother turbidity curves in these comparisons would indicate a smaller experimental error in turbidity measurements than in cell counts.

Frequent observation of aerated liquid cultures during a complete growth cycle will leave no doubt as to the occurrence of significant changes both in size and composition of the individual *Azotobacter* cell—changes equally as radical as those that have so frequently been recorded photographically for cultures grown under other conditions (e. g., photographs by den Dooren de Jong (*6*)). After reproduction ceases in aerated *Azotobacter* cultures the individual cell may continue to increase in size until it exceeds its original volume tenfold or more. Furthermore, the often recorded "increase in refractivity" or accumulation of "storage products" may also take place under these conditions to such an extent as to result in parts or even in the entire cell becoming almost opaque

Still a third factor may enter the optical picture in that some strains may bring about a marked accumulation of colloidal excretory products, so-called gums, which influence the optical properties of some cultures and not others, or the same culture at one time and not another. It is obvious from figures 2, 6, 7, and 9 that, as a result of the operation of some such factors, the turbidity curve does not necessarily follow the numbers curve after the logarithmic growth phase has been passed.

In addition to number of cells and turbidity, a third criterion was employed to measure growth in these studies, namely, the volume of cells. Obviously the actual volume of cells will depend upon the number of cells times size. Attention has just been called to the variation in size of the individual cell as influenced by reproduction and age. Another factor influencing the *apparent* volume is the extent to which packing is accomplished in making volumetric measurements. Even when the centrifugal force applied is constant, packing appears to be markedly influenced by other factors, such as gum formation and flocculation, which varied widely, not only between strains but for the same culture at different ages. The close similarity between the curves for number, turbidity, and volume in certain instances (figs. 2 and 8) indicates that volume may represent growth fairly accurately. In other instances the volume curves obviously bore little relation to growth and have been omitted.

Quantitative measurements of some byproduct of metabolic activity, such as nitrogen fixed, or of some nutritive material utilized, such as the uptake of oxygen, have been employed as indicators of growth. In these studies easily measured glucose was sometimes substituted for mannitol as the source of energy and quantitative changes in the glucose content of the cultures were recorded.

It was early noted that changes in the H^+ concentration of a culture, when the mildly buffered liquid medium employed contained ammonium sulfate, paralleled the disappearance (utilization) of the NH_4 ion. Since $pH = \log 1/H^+$ the pH curve of a culture wherein growth is taking place should be inversely related to NH_4 utilization, and hence to growth as represented by NH_4 utilization. It should be kept in mind, however, that in a nitrogen-free medium or in a medium containing fixed nitrogen in the form of KNO_3 the above

described relationship between growth and pH does not hold. In the latter case a decrease in the H^+ concentration parallels the utilization of NO_3 ions due to the excess of K ions (fig. 5).

In general the carbohydrate utilization curve, pH curve (inverted), and turbidity curve paralleled the cell-number curve so long as active cell division was taking place. After the cessation of active growth the close relationship between these various factors did not always continue. As a rule pH values continued to decrease, provided the supply of NH_3–N was not exhausted; cell counts also decreased, possibly as a result of flocculation or biochemical disintegration; and turbidity
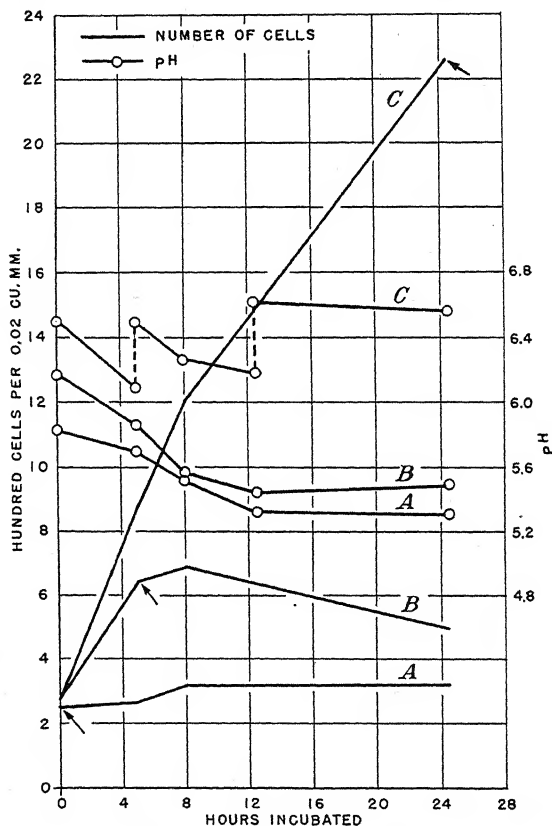
FIGURE 3.—Curves showing the influence upon growth of *Azotobacter* strain 33L of favorable pH levels maintained by neutralizing acidity with NaOH. *A*, *B*, and *C* received identical treatment except with respect to initial pH; broken portion of pH curve for *C* indicates readjustment of pH by addition of NaOH.

continued to increase. It is known from respiration studies on resting *Azotobacter* cells that many organic substrates may be utilized in appreciable quantities long after reproduction has ceased. Large quantities of glucose were observed to disappear from heavy suspensions of cells in the absence of any significant increase in the number of cells (figs. 4, A and B). This observation relative to glucose is not unexpected in view of the often recorded utilization of glucose by resting cells in respiration studies.

DISCUSSION

The data presented in table 1 indicate that the maximum H^+ concentration compatible with the growth of different strains of *Azotobacter* varies within the approximate limits, expressed as pH, of 5.5 and 6.0. However, as the maximum limiting H^+ concentration is approached growth is greatly retarded. The presence of combined nitrogen exerted no influence upon the critical H^+ concentration for growth.

The data presented graphically in figures 1 to 11 lend substantial support to a number of observations among which the following appear worthy of note.
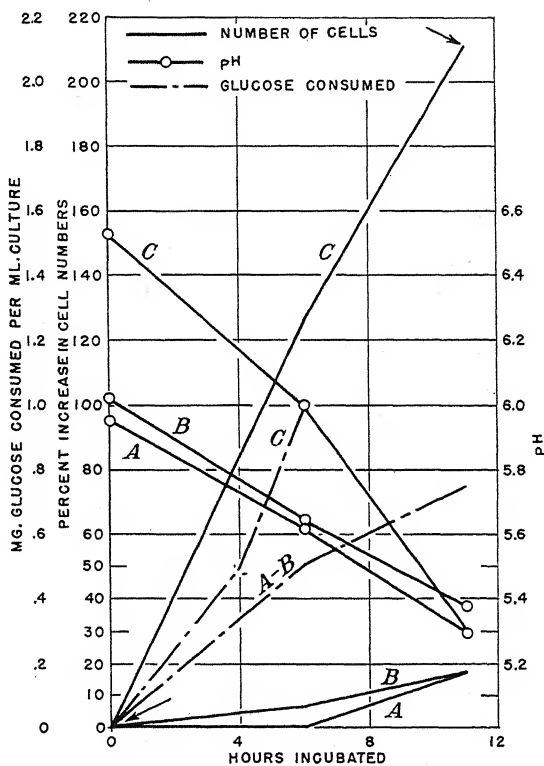
FIGURE 4.—Utilization of glucose by *Azotobacter* strain C_7b_4 in the presence of growth (*C*) and in the absence of growth (*A* and *B*) but in the presence of large numbers of cells. (Initial cell count 2,100 per 0.02 cu. mm.). Cell-number curve plotted on percentage increase in numbers.

The curves representing number of cells are characteristic of growth curves in general. In some instances, possibly because of inappropriate timing of analyses, the initial lag is not evident (figs. 3, 4, 8, and 9). The maximum number of cells was often reached in 4 to 6 hours (figs 7 and 9, *B*), and sometimes represented a very small total increase, not exceeding 5,000,000 per milliliter (fig. 5, B_2, B_3, and B_4). Retardation or cessation of active reproduction was: (1) independent of the number of cells, the maximum number of cells reached varying more than tenfold (compare curve *B* with *C* in figure 3, and curve B_5 with

B_2, B_3, and B_4 in figure 5); (2) not due to the exhaustion of the energy substrate (fig. 6); and (3) not a function of time, becoming operative within 4 to 6 hours (figs. 3, B; 7; and 9, B) or only after 48 hours (fig. 5, B_5).

Turbidity curves were of the same general form as those representing number of cells except that turbidity frequently continued to increase after cell division had ceased (figs. 6 and 7, and fig. 9, B) and sometimes developed in the absence of any reproduction. Quantitatively, however, the relationship between turbidity and number of cells varied for different cultures, the ratio cell numbers: turbidity being approximately seven times as great in one instance as in another. Turbidity may continue to increase for a time after cessation of re-
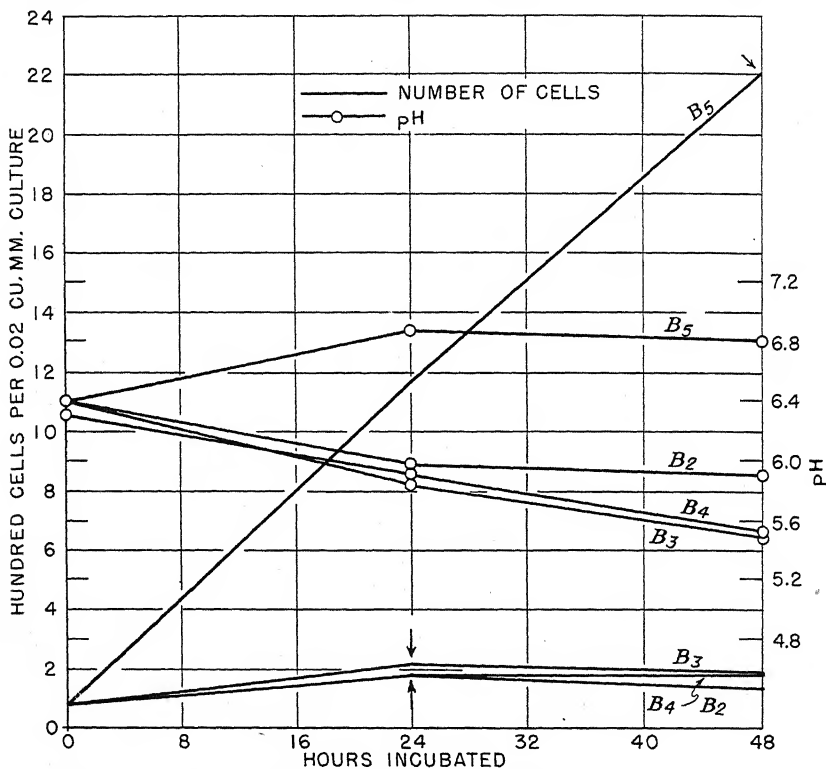
FIGURE 5.—Curves showing the influence upon growth of favorable (high) pH levels (curves B_5) maintained through absorption of the NO_3 ion when KNO_3 was present, *Azotobacter* strain 37b. B_2, B_3, and B_4 media contained $(NH_4)_2SO_4$; B_5 media contained KNO_3.

production at approximately the same rate as during the phase of rapid growth (figs. 6 and 7). This variation in relative turbidity may be explained on the basis of variation in size and composition of the cells of different strains, while the increase in turbidity in the absence of growth probably resulted from an increase in size and change in composition with increased age.

Where satisfactory volumetric measurements were obtained, curves plotted from the data were of the same general type as those for number of cells (figs. 2 and 8).

Changes in the pH value of a medium containing ammonium sulfate are inversely related to changes in cell number and turbidity, at least until active reproduction ceases (figs. 1 to 11). After the phase of active growth, however, the pH curve may continue to fall, even though the number of cells is constant or is decreasing (figs. 2; 5, B_3 and B_4; 7; and 9, B). The decrease in pH (increase in H^+) in such cases is due primarily to the absorption of NH_4 ions, hence is influenced by the original concentration of NH_4 (fig. 9), or by NH_4 subsequently added to the medium. Further decreases in H may cease altogether when the supply of NH_4 is exhausted (fig. 9, A) in spite of the fact that growth may continue. The absorption of NH_4 and the accompanying fall in pH may continue after reproduction has ceased (fig. 9, B) or may be markedly evident in the absence of growth provided the
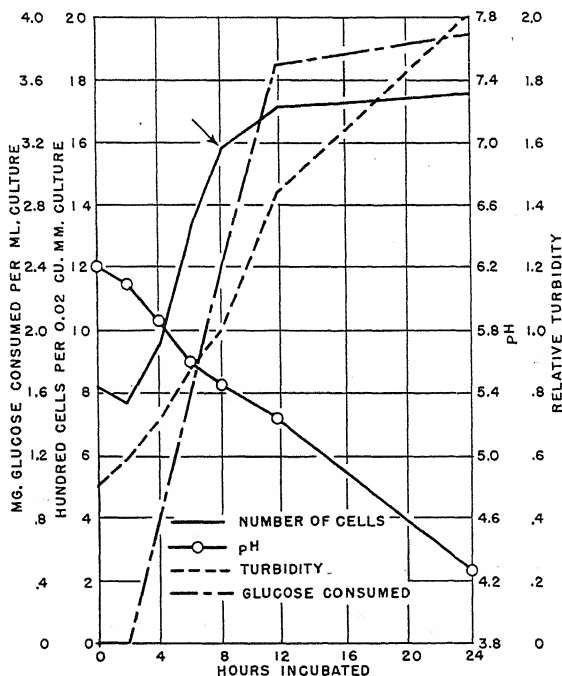
FIGURE 6.—Relation between growth and utilization of glucose by *Azotobacter* strain 13. Cessation of growth was not due to glucose exhaustion, the medium still containing 8.33 mg. of glucose per ml. of culture at the 24-hour analysis.

number of cells introduced in the initial inoculum is high enough to bring about quantitatively measurable changes (fig. 4, A, and B).

Curves based upon the quantity of energy substrate utilized tend to parallel growth curves where active reproduction is taking place (figs. 4, C, and 6); however, following a heavy inoculation under conditions unfavorable for cell division, relatively large quantities of glucose may be metabolized (fig. 4, A and B). The glucose consumption under such conditions may closely parallel the development of turbidity.

The general type of the growth curve (turbidity in this case) is similar, whether glucose, fructose, or mannitol is the source of energy (fig. 10).

As already suggested, cell-number and pH curves are inversely related during active growth. The higher the initial pH, within the limit of these studies, the higher the maximum number of cells reached (figs. 3, 4, and 11) and the longer the incubation period necessary before the maximum is reached.

If the initial H^+ concentration is above a maximum, varying between the pH values of 5.5 and 6.0 for different strains, no increase in cell numbers will take place and as the H^+ concentration approaches this maximum the rate of growth is greatly retarded (figs. 3 and 4).
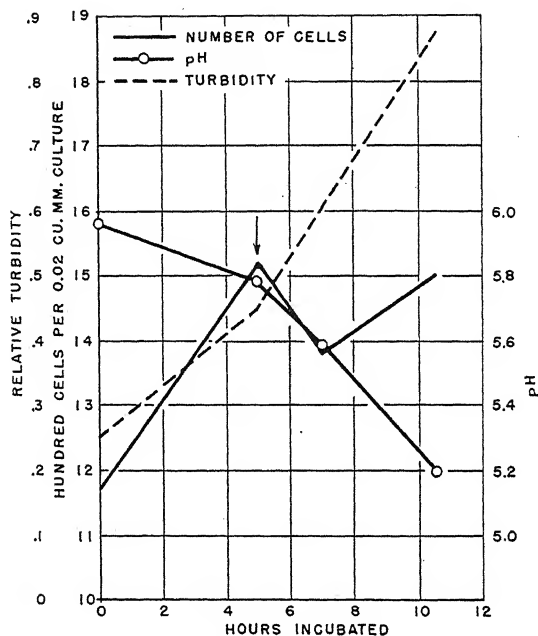
FIGURE 7.—Curves showing how cessation of growth of *Azotobacter* strain C_7–b_2 was followed by a marked increase in turbidity and utilization of fixed nitrogen. The original 10 p. p. m. of nitrogen added was exhausted after 5 hours, and an additional 5 p. p. m. disappeared during the following 2 hours without any accompanying increase in cell numbers.

If the initial H^+ concentration is such that active cell division can take place and as a result of this growth the H^+ concentration increases, as soon as it reaches the maximum for the particular strain in question further cell division will cease (figs. 1 to 11). On the other hand, if the necessary conditions exist for maintaining the H^+ concentration below the indicated maximum, growth will continue until limited by some other factor (figs. 3, 5, 9, and 11).
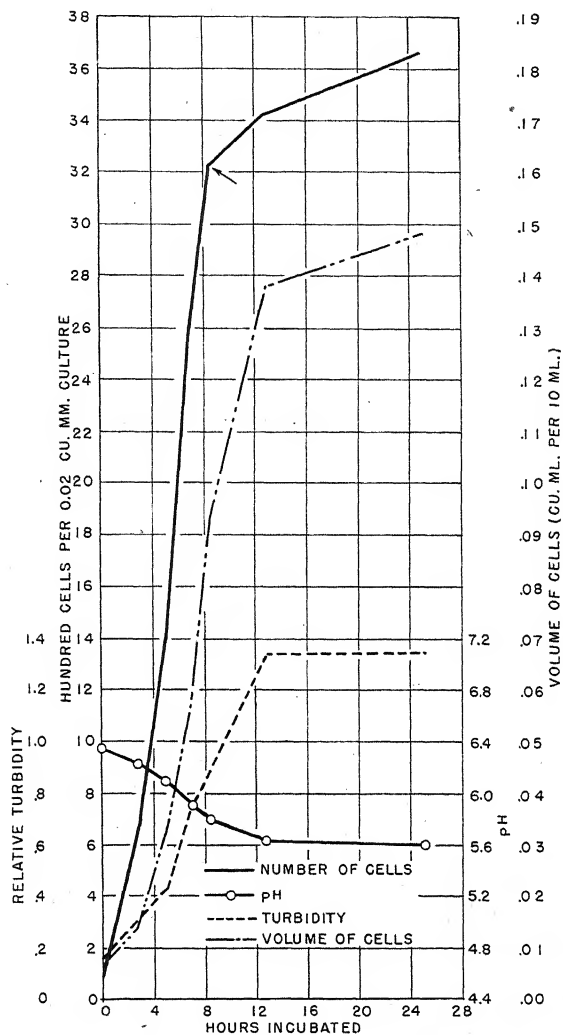
FIGURE 8.—Curves showing high bacterial numbers, *Azotobacter* strain C₇–b₃ accompanied by low turbidity.
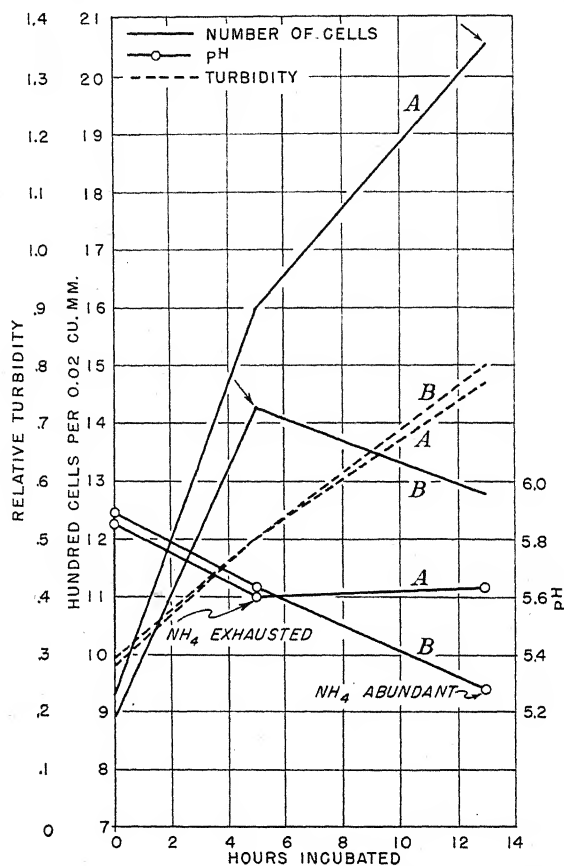
FIGURE 9.—*A* shows continued growth of *Azotobacter* after exhaustion of fixed nitrogen in medium when initial nitrogen content was 10 p. p. m. and pH was favorable. Nitrogen was exhausted at 5 hours, pH was constant thereafter, and growth continued. *B* shows how growth ceased when original nitrogen content was 25 p. p. m., nitrogen was abundant, and pH content continued to fall.
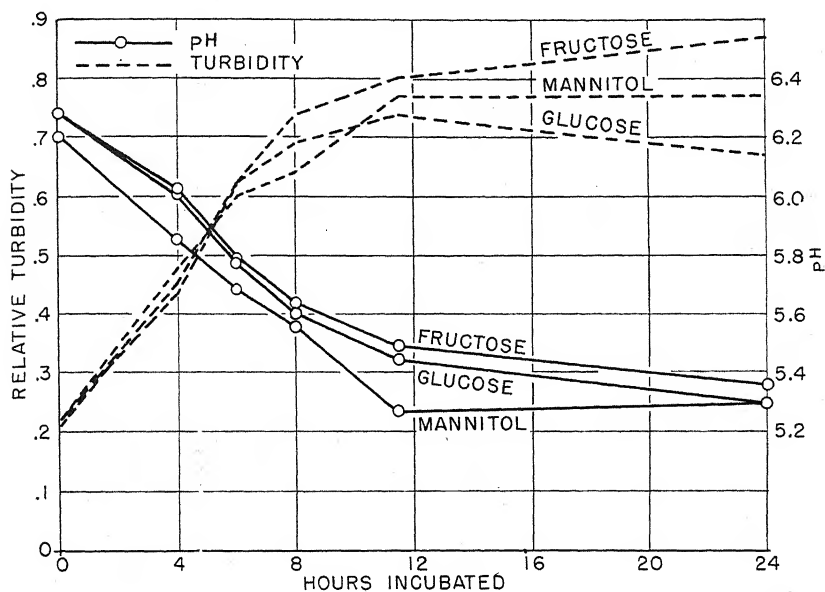
FIGURE 10.—Curves showing the influence of the source of energy upon growth (turbidity) of *Azotobacter* strain O₂.
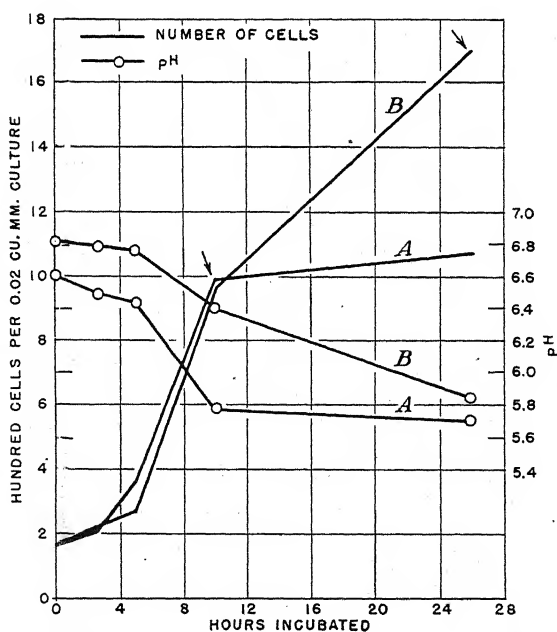
FIGURE 11.—Curves showing the influence upon growth of *Azotobacter* strain 22 of temporary maintenance of favorable pH by the addition of CaCO₃. The *B* culture is a duplicate of *A* except that it contained a small quantity of additional CaCO₃

SUMMARY

Data accumulated from an intensive study of more than 40 pure-cultures of *Azotobacter* warrant the conclusion that the H^+ concentration of the medium exercises a profound influence, either directly or indirectly, upon the growth (reproduction) of this organism.

The maximum H^+ concentration compatible with growth varies somewhat with different strains but in general falls within the limits, expressed as pH, of 5.5 and 6.0. Growth is markedly retarded within a few tenths pH unit of the critical H^+ concentration.

The presence of fixed nitrogen does not alter the critical H^+ concentration for any strain studied.

Increase in size of the individual cell and metabolization of fixed nitrogen and energy substrate can continue at H^+ concentrations inhibitory to cell division.

In a liquid medium culture of *Azotobacter*, during the period of active cell division, changes in turbidity, cell volume, energy substrate, and (in the presence of ammonium sulfate) ammonium nitrogen and H^+ concentration parallel changes in cell numbers; under such conditions, therefore, these changes may be taken as criteria of growth. In the absence of active cell division, however, any one or all of these factors may undergo radical alteration independent of growth.

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THE EFFECT OF SOME ENVIRONMENTAL FACTORS ON THE SET OF PODS AND YIELD OF WHITE PEA BEANS [1]

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INTRODUCTION

Approximately 87 percent of all white pea beans (*Phaseolus vulgaris* L.) grown in the United States are produced in Michigan, and 90 percent of the total acreage in Michigan is in 19 counties of the east-central part. The average yield of beans from this area is higher than from other areas of the State on soils of equal fertility. Experiments designed to increase the yield with commerical fertilizers have given inconsistent results. These experiments, carried out over a 21-year period by members of the Soil Science Section of the Michigan Agricultural Experiment Station, have shown a favorable early response of the crop to commercial fertilizer applications which would be maintained up to the blooming period, but often this apparent improvement in the growth of the crop was not reflected in the yield at harvest. In view of this situation, the following study was instituted in order to determine the effects of certain climatic factors on the development of the crop, especially during the critical period of pod formation. These experiments included a study of the effect of temperature, humidity, fertilizer, soil moisture, and leaf area on the set of pods and on the yield of the white pea bean both in the greenhouse and in the field.

PROCEDURE

The temperature and humidity measurements were secured from a Friez recording hygrothermograph which was placed at the level at which the plants were growing both in the greenhouse and in the field. Soil moisture was determined by drying the soil samples in an oven at 105° C. and calculating the percent of moisture from the resultant loss in weight.

GREENHOUSE EXPERIMENTS

In 1940 data on leaf area, yield, and set of pods were obtained from bean plants grown in 1-gallon jars in the greenhouse. The six treatments were as follows: A 4–16–8 fertilizer was applied to Miami loam at the rate of 600 pounds per acre at three soil-moisture levels, low, optimum, and high. The corresponding controls (low, optimum, and high moisture levels) received no fertilizer. The moisture levels were maintained by bringing the jars to original weight at frequent intervals. Two glass tubes, ¾ inch in diameter, were placed 1 inch and 5 inches, respectively, from the bottom of the jars. The capacity

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of these tubes was such that enough water could be added each day to keep the moisture content uniform throughout the jar. Three plants were grown in each jar and two replications of the six treatments were made. To extend the blooming period and so observe the plants under a greater variety of temperature and humidity conditions, the jars were planted at approximately 3-week intervals from April 6, 1940, to August 1, 1940. Whenever weather conditions permitted, the plants were removed to a wire-enclosed space adjacent to the greenhouse. The leaf area and yield of the individual plants were obtained, together with the set of pods and the temperature and humidity data as indicated by the hygrothermograph.

FIELD EXPERIMENTS

Field beans of the Michelite variety were planted in 28-inch rows in 1939, 1940, and 1941, on Miami loam at the Miller farm, Ovid, Mich. Plantings were made at approximately 5- to 7-day intervals for 6 weeks, from the last week in May to the first week in July. A 4-16-8 fertilizer at the rate of 500 pounds per acre was applied to one-half of the rows in a band 1 inch to the side of and 1¾ inches below the seed level. The hygrothermograph did not arrive in time to be used during 1939, and consequently only yield data from the different planting dates were taken in that year. In 1940 and 1941, just before blooming, plants were selected at random from the various treatments and the leaf area was determined according to the method described by Davis.[3] The number of leaf areas compiled depended on the amount of help available and the rapidity with which the plants came into bloom. The yields of both beans and straw of each plant was taken at harvesttime.

To determine the set of pods a small tag marked with the appropriate date was attached just below a pair of blossoms, and a few days later a count was made of the number of blossoms that formed pods.

Soil samples for moisture determination were collected at 2- to 3-day intervals throughout the blooming period.

In 1941 temperature and humidity data were taken at two locations in the bean-growing area, one of the locations supposedly more favorable for bean production than the other.

From the data taken the following correlations were made: Leaf area versus yield, yield of straw versus yield of beans, percent set of pods versus maximum temperature, percent set of pods versus minimum relative humidity, maximum temperature versus minimum relative humidity, percent soil moisture versus percent set of pods, percent set of pods versus maximum temperature with humidity held constant, percent set of pods versus minimum humidity with temperature held constant, and percent set of pods versus maximum temperature and minimum humidity combined. In addition, the effect of fertilizer on set of pods was calculated by analysis of variance, and the effect of date of planting and of fertilizer application on the yield of beans was obtained.

A prediction equation of temperature and yield was also calculated from the field data.

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FIELD EXPERIMENTS

RELATION BETWEEN SET OF PODS AND TEMPERATURE, SET OF PODS AND RELA-
TIVE HUMIDITY, AND MAXIMUM TEMPERATURE AND MINIMUM RELATIVE
HUMIDITY

The correlation coefficients and z values calculated for temperature and percent set of pods are reported in table 1. The values for r were calculated for six different factors, namely, percent set versus maximum temperature on day the blossoms were tagged; percent set versus average maximum temperature for the day the blossoms were tagged and for the following day; percent set versus the area under the curve as measured with a planimeter taken from the hygrothermograph chart with 60° and 70° used as bases for the measurements; the areas secured from both the 60° and 70° bases were correlated against percent set as area for the day the blossoms were tagged and also as the average area for the date the blossoms were tagged and the following day. The justification for using the area under the curve was based on the assumption that an intensity heat factor would be introduced which would result in a higher correlation between the percent set of pods than if maximum temperatures only were used. Since a relatively small number of items were correlated the r's were changed to z values [4] in order to determine whether or not any significant differences existed between the correlation coefficients calculated by the various methods. No significant differences were found, and the average maximum temperature for 2 successive days was later used in calculating the multiple and partial correlation coefficients.

The data in table 1 show a significant relation between the percent set of pods and minimum relative humidity.[5] However, minimum relative humidity does not exert as great an effect on the set of pods as does maximum temperature.

TABLE 1.—*Correlation coefficients and z values calculated between percent set of pods and maximum temperature, percent set of pods and minimum relative humidity, and maximum temperature and minimum relative humidity, Miller farm, 1940 and 1941*

Item	1940		1941		1940 and 1941	
	r	z value	r	z value	r	z value
Percent set of pods versus maximum temperature	−0.6107	−0.7100	−0.6576	−0.7886	−0.5962	−0.6872
Percent set of pods versus minimum relative humidity	.5126	.5662	.4698	.5098	.4653	.5042
Maximum temperature versus minimum relative humidity	−.4146	−.4417	−.4421	−.4747	−.4664	−.5055
Difference between z values required for significance	----------	.8104	----------	.7673	----------	.5070

The correlation coefficients between maximum temperature and minimum relative humidity were calculated for each of the 2 years 1940 and 1941 and for the combined data for the 2 years. The average

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[5] Minimum relative humidity values for the date the blossoms were tagged were used in calculating the correlation coefficients. If areas under the curve are used, a negative correlation coefficient is obtained, making this method unsuitable.

maximum temperature for 2 successive days was correlated with the minimum relative humidity for the date the blossoms were tagged. The data show that a significant correlation of -0.4664 exists between maximum temperature and minimum relative humidity for the years 1940 and 1941. The degree of relationship between maximum temperature and minimum relative humidity is not as great as between the percent set of pods and maximum temperature.

MULTIPLE AND PARTIAL CORRELATIONS BETWEEN PERCENT SET OF PODS, MAXIMUM TEMPERATURE, AND MINIMUM RELATIVE HUMIDITY

The multiple and partial correlation coefficients between the percent set of pods, maximum temperature, and minimum relative humidity for 1940 and 1941 follow:

$$Rx.yz \text{---------------------------------------} 0.6538$$
$$Rxy.z \text{--} -.5192$$
$$Rxz.y \text{--} .2523$$

x refers to the percent set of pods, y to the average maximum temperature for two successive days, and z to the minimum relative humidity for the date the blossoms were tagged.

The average maximum temperature for the 2 successive days and the minimum relative humidity for the date the blossoms were tagged were used in calculating both the multiple and the partial correlation coefficients. Two of the coefficients, $Rx.yz$ and $Rxy.z$, indicate a high degree of relationship between the factors mentioned. However, with temperature held constant the effect of humidity is not great enough to be significant, indicating that maximum temperature is the most important climatic factor affecting blossom development of the field bean.

PREDICTING PERCENT SET OF PODS FROM TEMPERATURE

A predicting line was calculated between percent set of pods and maximum temperature for 1940 and 1941. The equation follows:

Predicting equation ----------------------- $y = -1.8x + 192$
Standard error of prediction ------------------------- 3.82
Percent error of mean ------------------------------- 7.62

x refers to the average maximum temperature for 2 successive days and y to the percent set of pods.

The equation indicates that approximately 57 percent of the blossoms will set pods if the average maxiumm temperature for any 2 sucessive days during the blooming period does not exceed 75° F. For each degree of temperature above 75° a reduction of approximately 2 percent in the set of pods will result. However, in using a predicting equation of this kind based on one climatic factor it should be remembered that there are a number of inherent errors that might be encountered for any specific time. For example, other climatic factors that influence the behavior of plants, such as soil temperature, soil moisture, relative humidity, and light intensity, may decrease the accuracy of a single prediction factor. Another important fact that must be considered is the difficulty of estimating the lag in the time required for a change in temperature to manifest itself in plant development. In working with beans it has been observed that after a few days of high temperature some time is required for the plants to recover sufficiently to take full advantage of a period of optimum temperature for set of pods. Another situation in which the

correlation between maximum temperature and percent set of pods is affected occurs when a day of optimum temperature comes during a period of high temperatures. In such cases the full advantage of an increase in the percent set of pods that would be expected if temperature were the only factor concerned is not attained.

In addition, strict linearity of the data is assumed, and in this respect it should be pointed out that this equation is applicable only within the range of temperature of 55° to 98° encountered in this investigation. Predicting the percent set of pods from temperatures outside this range could easily lead to erroneous results.

TEMPERATURE AND RELATIVE HUMIDITY VARIATIONS WITH RESPECT TO LOCALITY

The data in table 2 show that during the blossoming period of 1941 the average maximum daily temperature was significantly lower and the average minimum daily relative humidity significantly higher at the Horst farm, located in the best bean-growing area, than at the Miller farm, located just outside of this area. Although these data are for only one year, they are based on daily records for two different periods, from July 14 to August 31 and from July 14 to August 17. The July 14–August 17 period is the time in which most of the pods are formed, and comparisons made between temperature and humidity conditions at the two locations during this critical period should give more practical information regarding climatic effects than if the data for the July 14–August 31 period alone were used. The mean differences for both temperature and minimum humidity for the Miller farm are significant at the 1-percent level in all cases except one, that is, the mean difference for relative humidity for the July 14–August 31 period, and in this case the measure of significance is well above the 5-percent level. Since it has been shown that after the maximum temperature has reached 75° F. the percent set of pods rapidly decreases and that there is also some relation between percent set of pods and minimum relative humidity, it follows that an area in which lower maximum daily temperature and higher minimum relative humidity prevail would be more favorable for this crop provided soil conditions are comparable. The assumption is borne out by the facts of the case since the Horst farm is in the center of the best bean-growing area in the State and the Miller farm is outside of this area.

TABLE 2.—*Mean differences between daily maximum temperature and minimum relative humidity at the Miller and Horst farms in 1941*

Location [1]	Maximum temperature				Minimum relative humidity			
	July 14–Aug. 31		July 14–Aug. 17		July 14–Aug. 31		July 14–Aug. 17	
	Mean	Mean difference	Mean	Mean difference	Mean	Mean difference	Mean	Mean difference
Miller farm	80.43	3.79*	82.17	3.54*	29.86	2.46**	27.83	3.54*
Horst farm	76.64		78.63		32.32		31.37	

[1] The Horst farm at Akron, Mich., is located in the most favorable bean-growing area and the Miller farm at Ovid, just outside of this area.
* Significant at the 1-percent level.
** Significant at the 5-percent level.

INFLUENCE OF SOIL MOISTURE ON SET OF PODS

The correlation coefficients calculated between percent set of pods and soil moisture for the Miller farm for 1940 and 1941 are shown in the following tabulation:

Percent set of pods versus percent moisture in surface soil_____[1] 0.3482
Percent set of pods versus percent moisture in subsoil_____ .1913

According to these data neither the percent of moisture in the surface 6 inches of soil nor in the subsoil had any significant effect on set of pods.

INFLUENCE OF FERTILIZER ON SET OF PODS

To determine the effect of fertilizer on set of pods a 4–16–8 fertilizer was applied at the rate of 500 pounds per acre in a band 1 inch to the side and 1¾ inches below the seed in 1940 and 1941. The results, presented in table 3, show no apparent influence of fertilizer on set of pods in either season. However, the difference in the percent set of pods on the various dates was highly significant, indicating again the role of climatic factors in the production of the field bean. Table 4 presents the results of an analysis of the variance of yield of beans, as shown in table 3.

TABLE 3.—*Effect of fertilizer on set of pods, Miller farm,[1] 1940 and 1941*

	Percent set in 1940				Percent set in 1941		
Date	Ferti-lized	Unferti-lized	Weighted mean [2]	Date	Ferti-lized	Unferti-lized	Weighted mean [2]
July 23_____	10.0	2.5	3.5	July 14_____	67.1	52.6	60.2
July 24_____	10.6	6.7	8.4	July 16_____	80.1	69.3	74.4
July 26_____	18.8	12.1	15.8	July 18_____	91.8	85.9	89.7
July 29_____	11.1	12.4	11.8	July 21_____	78.9	71.2	74.1
July 30_____	5.1	6.5	5.7	July 23_____	75.5	65.6	69.8
August 2_____	22.1	24.7	23.2	July 25_____	38.6	27.3	32.9
August 5_____	50.7	49.7	50.2	July 28_____	19.7	18.9	19.5
August 7_____	51.8	67.6	59.3	July 30_____	39.1	23.9	36.5
August 10_____	72.5	74.8	73.5	August 1_____	21.7	25.7	22.8
August 12_____	74.1	72.5	73.4	August 4_____	13.0	14.6	13.5
August 14_____	46.3	54.3	51.9	August 6_____	4.3	15.0	9.0
August 16_____	73.9	81.3	77.1	August 8_____	16.2	20.1	13.6
August 19_____	63.3	64.0	63.6	August 11_____	53.9	55.5	52.4
August 20_____	68.4	76.5	73.8	August 13_____	29.1	50.0	37.2
August 23_____	53.3	78.9	66.3	August 25_____	72.8	73.4	73.2
August 31_____	89.8	84.8	87.3	August 28_____	73.1	58.3	72.2
September 3_____	94.0	92.3	93.1				

[1] The percent set of pods is based on 21,036 blossoms in 1940 and 14,508 in 1941, a total of 35,544 blossoms for the 2 years.
[2] Means weighted according to number of blossoms counted.

TABLE 4.—*Analysis of variance of set of pods shown in Table 3*

Source of variance	1940			Source of variance	1941		
	Degrees of free-dom	Sum of squares	Mean square		Degrees of free-dom	Sum of squares	Mean square
Total_____	33	30,817.24	_____	Total_____	31	21,339.30	_____
Dates_____	16	30,190.56	1,886.91*	Dates_____	15	20,496.60	1,366.44*
Fertilizer_____	1	61.70	61.70	Fertilizer_____	1	70.81	70.81
Error_____	16	565.18	35.51	Error_____	15	771.89	51.46

*Significant at the 1-percent level.

[1] A value of 0.3976 is required for significance at the 5-percent level.

INFLUENCE OF FERTILIZER AND DATE OF PLANTING ON YIELD

The data in table 5 show that a 4–16–8 fertilizer applied at the rate of 500 pounds per acre to a bean crop in 1939 significantly increased the yield regardless of the planting date and that the yields for both the earliest and latest planting dates, May 31 and June 12, respectively, were significantly higher than those from either of the plantings made on June 3 or June 9. The yield from the planting made on June 6 was not significantly higher or lower than any of the other yields. The lack of any definite trend in yields and planting dates, although there is a direct relation between date of planting and time of blossoming, further indicates the possibility that differences in yield from different dates of planting are due to the weather conditions prevailing during the blooming period. Table 6 presents the results of an analysis of the variance of yield of beans, as shown in table 5.

TABLE 5.—*Effect of fertilizer and date of planting on yield of beans, Miller farm, 1939*

Planting date	Yield per acre [1]		Planting date	Yield per acre [1]	
	Fertilized [2]	Unfertilized		Fertilized [2]	Unfertilized
	Bushels	*Bushels*		*Bushels*	*Bushels*
May 31	14.0	8.9	June 9	10.3	5.6
June 3	10.4	6.4	June 12	12.7	7.0
June 6	11.9	6.3			

[1] Average of 4 replications.
[2] Fertilized at rate of 500 pounds of 4-16-8 fertilizer per acre.

TABLE 6.—*Analysis of variance of yield of beans, as shown in table 5*

Source of Variance	Degrees of freedom	Sum of squares	Mean square	Source of variance	Degrees of freedom	Sum of squares	Mean square
Total	39	533.77	-------	Fertilizer	1	248.56	248.56**
Blocks	3	117.65	39.21	Fertilizer × dates	4	3.94	.99
Dates	4	60.58	15.14*	Error (b)	19	50.24	2.64
Blocks × dates (a)	12	52.80	4.40				

* Significant at the 5-percent level.
** Significant at the 1-percent level.

INFLUENCE OF FERTILIZER ON LEAF AREA AND YIELD OF BEANS AND STRAW

The data in table 7 show that a 4–16–8 fertilizer applied at the rate of 500 pounds per acre caused a significant increase both in leaf area and in yield of straw and beans in some cases, but these increases were more consistent in 1941 than in 1940. In 1940, in only one case in five did a significant increase in either the yield of beans or of straw result from an application of fertilizer. However, in three cases in five a significant increase in leaf area was observed. In all cases in 1941 significant increases in yield of straw and in leaf area resulted from a fertilizer application, and in three out of four cases the yield of beans was significantly better. These data indicate a considerable seasonal effect of fertilizer on plant behavior. It is interesting to note that the significant differences found in 1940 were significant only at the 5-percent level, whereas in 1941 all significant

differences were at the 1-percent level. This situation may be partly due to the greater competition between plants in 1940 resulting from closer spacing in that year. In 1940 the plants were thinned to approximately 4 inches apart and in 1941 to 8 inches apart.

The effect of date of planting on the leaf area and on the yield of both straw and beans is noted in the wide variation between the values for the different planting dates. In 1940 the leaf area ranged from 201.2 to 474.4 square inches for plants receiving fertilizer and from 154.9 to 406.2 square inches for plants not receiving fertilizer. Similar variation in both the yield of beans and straw were found. The variations in leaf area and in yields of beans were not as wide in 1941 as in 1940, again emphasizing the effect of the season.

TABLE 7.—*Mean differences of yield of beans, leaf area, and yield of straw between fertilized and unfertilized bean plants, Miller farm, 1940 and 1941* [1]

1940

Date planted	Yield of beans	Leaf area	Yield of straw
	Grams	*Sq. In.*	*Grams*
June 8	5.1±9.4	54.5±33.6	4.6±6.2
June 11	−1.7±6.1	71.6±22.5*	3.6±4.3
June 14	18.1±5.7*	83.1±20.0*	12.9±4.7*
June 17	−3.0±4.9	46.3±14.8*	−1.7±2.8
June 25	3.7±4.3	68.2±48.5	6.0±4.1

1941

Date planted	Yield of beans	Leaf area	Yield of straw
June 3	7.5±6.7	166.0±33.9**	28.7±4.9**
June 9	13.4±2.2**	163.9±23.5**	17.3±2.3**
June 16	9.8±2.2**	163.7±26.4**	11.9±1.8**
June 24	7.6±1.9**	142.2±19.8**	9.3±1.6**

* Significant at the 5-percent level.
** Significant at the 1-percent level.
[1] A minus sign preceding a yield figure indicates that yield of fertilized plants was below that of unfertilized plants.

LEAF AREA AND YIELD RELATIONSHIPS

The data in tables 8 and 9 show the relation between leaf area and yield of beans and between weight of straw and yield of beans for individual plants grown in the field in 1940 and 1941. The correlation coefficients for the various planting dates may be either significantly positive or significantly negative, depending on weather conditions during the blossoming period. These coefficients show that little correlation exists between leaf area and yield of the field bean. The relation between yield of beans and yield of straw is, on the other hand, is highly significant for each planting date in 1940 and for five out of eight in 1941, indicating that the weight of straw is a better measurement of the yield of beans than the leaf area.

Fertilizer does not seem to have any consistent effect on the relation of leaf area and yield of beans or weight of straw and yield of beans.

TABLE 8.—*Correlation coefficients calculated between leaf area versus yield of beans and weight of straw versus yield of beans, Miller farm, 1940*

FERTILIZED [1]

Date of planting	Date set of pods was determined	Leaf area versus yield of beans	Weight of straw versus yield of beans
June 8	July 16–Aug. 14	0.5522*	0.9403*
June 11	July 22–Aug. 14	.3726	.7859**
June 14	July 30–Aug. 16	−.7366*	.8989**
June 17	July 30–Aug. 20	−.2746	.9121**
June 25	Aug. 16–Aug. 23	.4500	.6703**

UNFERTILIZED

June 8	July 23–Aug. 14	0.6044*	0.9674**
June 11	July 24–Aug. 19	.3853	.8693**
June 14	July 29–Aug. 12	.3127	.7867**
June 17	July 30–Aug. 20	.2922	.6733**
June 25	July 16–Aug. 23	.2800	.6474**

* Significant at the 5-percent level.
** Significant at the 1-percent level.
[1] Bean plants were fertilized at the rate of 500 pounds per acre of 4–16–8 fertilizer.

TABLE 9.—*Correlation coefficients calculated between leaf area versus yield of beans and weight of straw versus yield of beans, Miller farm, 1941*

FERTILIZED [1]

Date of planting	Date set of pods was determined	Leaf area versus yield of beans	Weight of straw versus yield of beans
June 3	July 14–July 28	0.5381	0.0579
June 9	July 23–Aug. 13	.5015*	.1732
June 16	July 30–Aug. 11	.5488*	.5474*
June 24	Aug. 22–Aug. 28	.1539	.6169**

UNFERTILIZED

June 3	July 14–July 30	0.5843	0.3956
June 9	July 30–Aug. 13	.4526*	.8850**
June 16	Aug. 6–Aug. 11	.2026	.6077**
June 24	Aug. 22–Aug. 28	.4735*	.7969**

* Significant at the 5-percent level.
** Significant at the 1-percent level.
[1] Bean plants were fertilized at the rate of 500 pounds per acre of 4–16–8 fertilizer.

GREENHOUSE EXPERIMENTS

The results obtained in the greenhouse differ in some respects from those obtained in the field. These differences may be briefly summarized as follows: Individual plants grown in the field had on an average 31 times more beans and 7 times more leaf area than plants grown in the greenhouse, and the ratio of beans to straw varied widely in plants grown under greenhouse and field conditions. Contrary to the results obtained in the field, no significant correlation was found between maximum temperature and percent set of pods or minimum relative humidity and percent set of pods in the greenhouse. However, the correlation between maximum temperature and minimum relative humidity under greenhouse conditions was found to be significant. In view of the results of this study, the assumption that

results obtained in the greenhouse in an investigation of this nature are applicable under field conditions is open to question.

DISCUSSION OF RESULTS

Among the factors that influence the development of the field bean plant, in addition to those studied, are wind velocity, amount, distribution, and intensity of rainfall, degree of cloudiness, and plant cover. These factors are especially important during the blossoming stage. A short discussion of the possible ways in which they may influence the bean plant, together with a few remarks on the growth habit of the plant, may help to explain some of the results obtained.

The recommended planting date for beans in Michigan is from June 1 to 10, and harvest begins approximately 3 months later. Blossoming begins 6 to 7 weeks after planting, starts at the lower part of the plant, progresses toward the tips of the branches, and extends to the runners if weather conditions are favorable. The plants may blossom, set pods, and continue to bloom for as long as 5 or 6 weeks if a wet period occurs late in the season. Thus there are ripe pods present and blossoms forming at the same time. In case of an early frost many of the immature pods formed during this late blooming period will be damaged and the quality of the crop will be appreciably lowered. However, the length of the blooming period makes it possible to get a fair yield even though weather conditions during part of this period are unfavorable.

It has often been observed that during days of high temperature and low relative humidity, if the wind velocity is high, a much lower set of pods is obtained than under similar conditions of temperature and humidity with wind velocity low. In fact, farmers associate these hot, dry winds with blasting of the blossoms. Wind velocity, then, can be a contributing factor in the set of pods and thus modify the effect of temperature alone.

Rainfall is also an important factor as, in addition to supplying soil moisture, it exerts a mechanical effect on blossom development. During heavy rains, the blossoms on the tips of branches or runners are driven into the ground and thus the set of pods is reduced. When the surface of the soil remains moist for long periods many of the blossoms that touch the ground rot and thus the set of pods is further reduced. It is apparent, therefore, that intensity and amount of rainfall affect the percent of pods independently of temperature, and so reduce the degree of correlation between temperature and set of pods.

The degree of cloudiness exerts a slight effect on the set of pods by modifying the effect of temperature and light. For example, if the maximum temperature is used in correlating temperature and percent set of pods, then during a cloudy day the length of time that the maximum temperature would affect the plant would be less than during a clear day with the same temperature, and the percent set of pods for each day would be different.

Temperature is the climatic factor that exerts the strongest influence on the percent set of pods. According to the results obtained, a significant correlation exists between these two factors, and the percent set of pods can be predicted with a fair degree of accuracy from maximum temperatures. The error for any single predicted value is 7.6 percent of the mean. This error is quite large, but in

consideration of the fact that temperature is only one of the factors involved, the magnitude of the error is well within the expected. This demonstrated effect of temperature shows the practicality of this type of study for determining whether or not any particular location would be suitable for the production of field beans. Significant daily temperature differences found in 1941 between two farms, one located in the typical bean area and the other just outside this area, add considerable weight to the data since here is a practical demonstration of conditions actually existing in the field for which a plausible explanation can be offered.

The inconsistency of annual response of the field bean to fertilizer can be explained largely as a result of the influence of temperature.

FIGURE 1.—The bean plants on the left were planted 1 week earlier than those on the right and blossomed during a more favorable period for setting pods. The plants on the left are well podded, whereas those on the right are practically devoid of pods.

In spite of the fact that the fertilizer stimulates vegetative growth, yield will be poor unless the temperatures prevailing during the blooming period are favorable. It has often been observed that fertilizer will hasten the blooming period just enough to cause the majority of blossoms to form during a hot, dry period and consequently there will be no increase in yield from the use of the fertilizer. The effect of date of planting on yield is illustrated by the plants shown in figure 1. There was just 1 week's difference in the date of planting, yet the plants in one case are well podded and in the other they are practically devoid of pods.

As compared with the effect of maximum temperature on the percent set of pods the effect of minimum relative humidity is of minor

importance, although a significant correlation was found between these two factors. In general, high maximum temperature is associated with low relative humidity, but this association was not found to be significant under the field conditions encountered except when the 2 years' data were combined. However, it was found that the average minimum relative humidity during the blooming period was significantly higher on a farm located in the typical bean-growing area than on another farm outside of this area, which suggests that humidity might have influenced the results.

Set of pods was not found to be associated to any appreciable degree with soil moisture changes. However, the data should be interpreted as meaning that the amount of moisture found in the soil did not reach a critical point either from the standpoint of an excess or a too limited supply. In other words, the data apply only within the limits encountered in the experiment because it is selfevident that any portion of a plant will not develop naturally if there is either an excess or a deficiency of moisture.

In studies of this nature, it is essential that the weather-recording instruments be placed in the area in which the experiment is being conducted and at the same level as that at which the plants are growing. Weather data supplied by a weather station even in the general vicinity cannot be depended upon to give a true picture of conditions prevailing at the experimental location. The modifying effect of the plant cover is not accounted for in weather data obtained in other than the same location as that in which the experimental plants are growing.

It might be assumed that the extent of leaf area of a plant would influence the yield since the ability of a plant to manufacture food increases with greater leaf area. However, the data obtained in this study do not show that this relationship is at all constant. Depending on the weather conditions at the time of pod formation, the correlation between leaf area and yield may be either positive or negative. This situation, then, implies the existence of other factors that influence the ratio of the yield of seed to leaf area. As the plants were grown within a limited area of the same soil, thus excluding the factor of soil fertility, the factors remaining must of necessity be climatic. The data show the temperature during the blossoming period to be the most important climatic factor involved.

SUMMARY AND CONCLUSIONS

The effects of temperature, humidity, soil moisture, leaf area, and fertilizer on the behavior of the white pea bean (*Phaseolus vulgaris* L.) were investigated both in the greenhouse and in the field. From the field studies the following conclusions were reached:

(1) Maximum temperature influences the set of pods more than any other of the factors studied and the percent set of pods can be predicted from maximum temperature with a fair degree of accuracy.

(2) Minimum relative humidity and soil moisture, within the limits encountered in the work, exert only a minor influence on the set of pods.

(3) The relation of leaf area to yield of beans may range from a positive to a negative correlation depending on the weather pre-

vailing during the blooming period. The yield of beans therefore, cannot be safely predicted from leaf area.

(4) Fertilizer had no effect on set of pods.

The response to environmental factors of plants grown under greenhouse conditions differed in certain important respects from that of plants grown in the field.

A study of the nature of the one herein reported is valuable for determining what areas may be best suited for crops that, like the white pea bean, have critical environmental requirements.
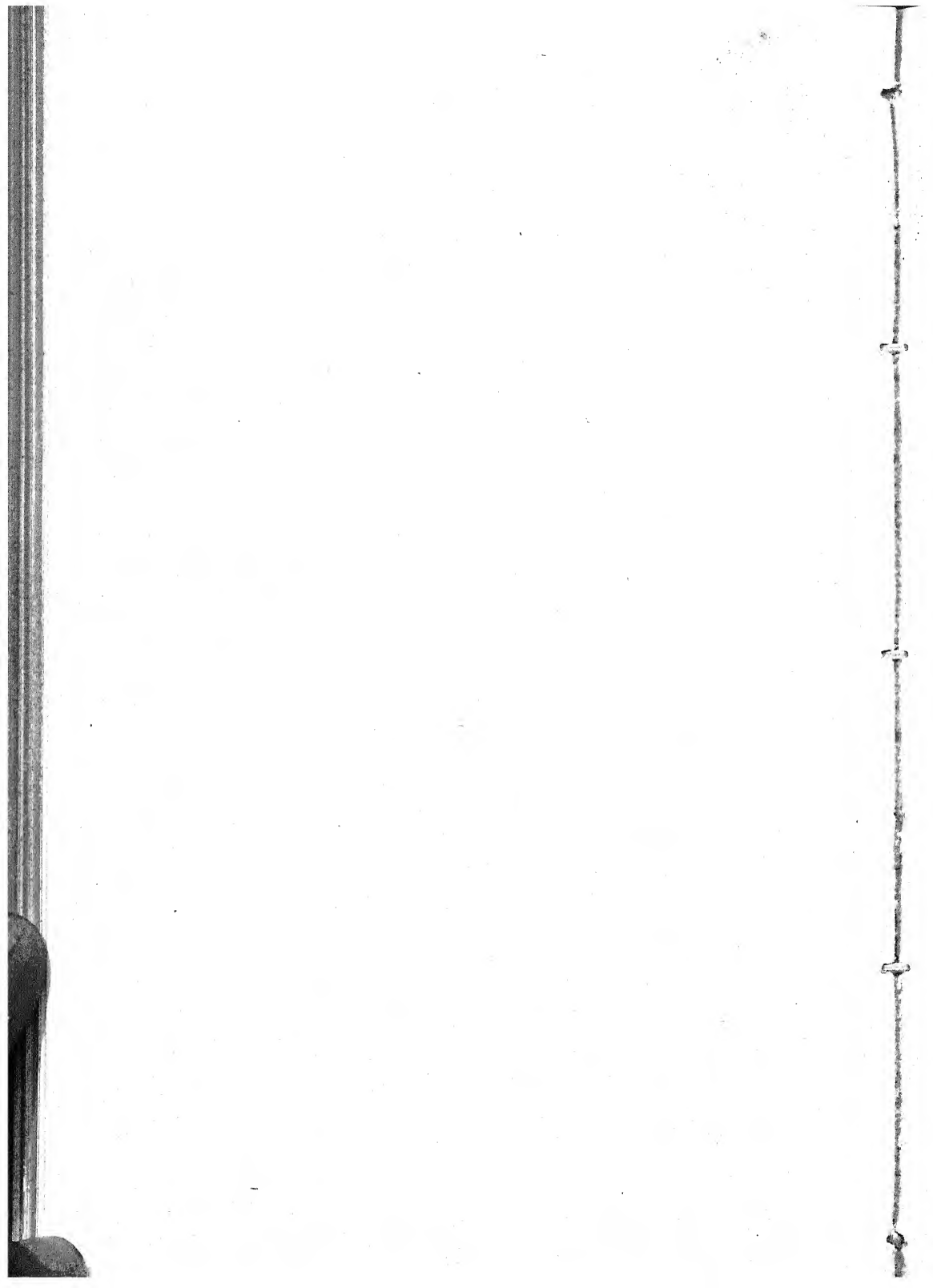
INHERITANCE OF NUMBER OF SEEDS PER POD AND LEAFLET SHAPE IN THE SOYBEAN [1]

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INTRODUCTION

Number of seeds per pod has been shown by Woodworth (11)[3] to be one of the several characters which contribute to yield of seed in the soybean (*Soja max* (L.) Piper). A clear understanding of the inheritance of these characters, as well as of the relations between them, is necessary in order to ascertain more accurately the possibility of obtaining all of them in the highest degree in one variety. This paper reports a study of the inheritance of number of seeds [4] per pod and leaflet shape. The latter character was included because of certain evidences of linkage between it and number of seeds per pod.

Soybean pods contain from one to five seeds. Plants of a given strain have either a majority of one-, two-, three-, or four-seeded pods or large percentages of any two consecutive classes from one to four. Strains with a majority of one-seeded pods are referred to in this paper as one-seeded strains, those with a majority of two-seeded pods as two-seeded strains, etc.

The terminal leaflet of most soybean varieties is ovate in shape; a few varieties have lanceolate leaflets, and one variety is known with oval leaflets (fig. 1). The lateral leaflets, although of the same general shape as the terminal leaflets, are somewhat asymmetrical and therefore have not been considered in this study.

REVIEW OF LITERATURE

Nagai (3) reported a cross between the three-seeded variety, Kaimame, and the two-seeded variety, Akutsuka. Approximately 70 percent of the pods of the F_1 were two-seeded, and about 70 percent of the F_2 plants resembled the two-seeded parent.

Data through the F_4 generation are presented by Takahashi (6) on a cross between Wearucong, a two-seeded variety with normal leaflets, and Yanta, a three- and four-seeded variety with narrow leaflets. A good fit to a 3:1 ratio for number of seeds per pod was obtained when the F_2 plants were divided into two classes according to whether less than 10 percent or more than 10 percent, respectively, of the pods from a given plant were four-seeded. Takahashi con-

[3] Italic numbers in parentheses refer to Literature Cited, p. 267.

[4] Includes aborted seeds and aborted or unfertilized ovules since these were considered potential seeds.

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cluded that the parents differed by a single gene and assigned to that allelomorphic pair the symbols F and f. The F_2 segregated in a 3:1 ratio of normal- to narrow-leaflet plants as Takahashi and Fukuyama (*7*) and Woodworth (*11*) had noted. Takahashi and Fukuyama (*7*) assigned the symbols Na and na to the allelomorphic genes involved. Linkage between narrow leaflet and high seeds-per-pod value was apparent in the F_2 and later generations, and the ratios obtained could be explained on the basis of 10 percent crossing over.

MATERIALS AND METHODS

The mean seeds-per-pod values of parental types available for crosses in this study ranged from 1.05 ± 0.01 for type 122 [5] to 3.59 ± 0.03 for type 114. With the exception of types 114, 122, 173, and 174, all of the types had seeds-per-pod means which tended to be somewhat intermediate between the extreme types, yet all of these means differed
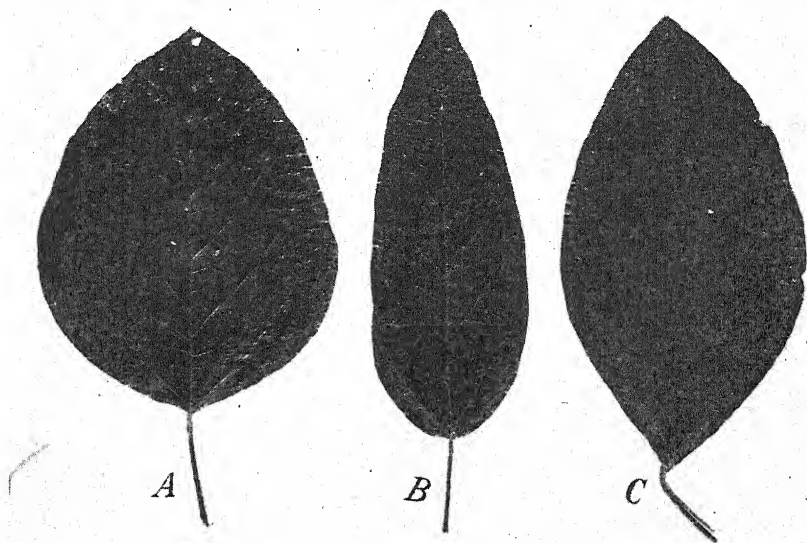
FIGURE 1.—Terminal leaflets of soybean plants: *A*, ovate or normal; *B*, lanceolate or narrow of types 114, 173, and 174; and *C*, oval of type 122.

significantly one from the other. These types are all classed as intermediate in this report. Types 173 and 174 had seeds-per-pod means which approached that of type 114. Types 114, 173, and 174 were therefore classed as high in seeds-per-pod value. Type 122 was classed as low. All of the intermediate types had normal leaflets, all of the high types had narrow leaflets, and the low type had oval leaflets.

Twenty crosses and six of their reciprocals were carried through the F_2 generation. Individual plants of the parental, F_1, and F_2 populations were classified for seeds-per-pod value and leaflet shape. A few progenies from two crosses were carried through F_3. Most of the F_1

[5] In accordance with Illinois Agricultural Experiment Station procedure, the strains used as parents have each been given a type number and the crosses have each been given a cross number.

values were obtained from plants grown under greenhouse conditions in gravel subirrigated with a nutrient solution. Because of the unusual environment in which these F_1 plants were grown, their seeds-per-pod values are not considered comparable to the parental and F_2 values obtained from field-grown plants.

The seeds for the F_2 were spaced at 4-inch intervals in consecutive rod rows 2 feet apart. Each F_2 population was bordered on one side by one row of one parent and on the other side by one row of the other parent. After harvest all pods were pulled from the plants and the number of one-, two-, three-, four-, and five-seeded pods was recorded for each plant. The number of potential seeds for each plant was obtained by summating the number of one-seeded pods multiplied by one, the number of two-seeded pods multiplied by two, etc. A seeds-per-pod value for each plant was calculated by dividing the total number of potential seeds by the total number of pods.

Leaflet-shape classifications of all populations were made by observation when the plants had reached approximately their maximum height. Difficulty was encountered in classifying some of the F_2 segregates. Several measurements were made on leaflets of parental and F_2 plants. Since the parents differed significantly in shape indices it was expected that the F_2 frequency distributions of these indices would be discontinuous and therefore classification would be facilitated. The measurements were made on five different terminal leaflets of each plant of the population at approximately equal intervals from the base of the plant to the top. The indices obtained on parental types and their F_2 generations segregating for leaflet shape were (1) the width of the leaflet at its widest point divided by its length, (2) the distance from the base of the leaflet to its widest point divided by its total length, and (3) the size of the angle formed by the two margins of the basal portion of the leaflet. The five values obtained on one plant for one index were averaged to give one index for the plant.

Twenty-five seeds from each of 25 plants were selected at random from the F_2 of a cross of intermediate-normal $\times$ high-narrow, along with 25 seeds of each parent. The seeds were planted in the manner described above to permit study of F_3. Seeds-per-pod and leaflet-shape classifications were made on these populations. Correlation coefficients between seeds-per-pod values of F_2 plants and their corresponding F_3 means and coefficients of variability were calculated.

Additional data on the inheritance of leaflet shape were obtained from 20 F_3 progenies from 12 narrow-leaflet and 8 oval-leaflet segregates of a narrow $\times$ oval cross.

Several of the F_2 populations yielded data for linkage tests between leaflet-shape genes and genes for other qualitative characters.

Since no difference between reciprocal crosses could be detected in any case, the data for reciprocals were combined for analysis and the two crosses identified as one. For example, crosses 516 and 524 were reciprocal crosses and are identified together as 516-524.

INHERITANCE OF NUMBER OF SEEDS PER POD

The relative constancy of seeds-per-pod values within a variety suggests that the character is governed largely by genic action. Weatherspoon and Wentz (9) counted seeds in 5 pods from each of 2 plants from 10 replications of 237 varieties. From the amount

of variance due to replications they concluded that the number of seeds per pod was influenced only slightly by soil differences. However, certain of the data obtained in the current study indicate that the character is not wholly uninfluenced by environmental factors. The planting of parental varieties in close proximity to each of their F_2 progenies necessitated planting 9 of the 11 varieties at more than one location in the field. Types 34, 38, 48, 114, 141, and 173 occurred twice in the field, type 10 occurred 3 times, and types 122 and 174 each occurred 4 times. When the t test was applied to all possible comparisons between seeds-per-pod means of the different plantings of each variety, 6 of the 21 mean differences were found to be significant at the 5-percent level. The significant differences were between the means of the 2 plantings of types 34, 38, and 114, and between 1 planting of type 122 and the other 3 plantings of type 122.

<center>INTERMEDIATE × LOW</center>

Four F_2 populations were derived from crosses between the one-seeded strain and the strains with intermediate values. Their F_2 frequency distributions were strikingly similar. Figure 2 shows the parental and F_2 distributions of cross 500, which is typical of this type of cross.
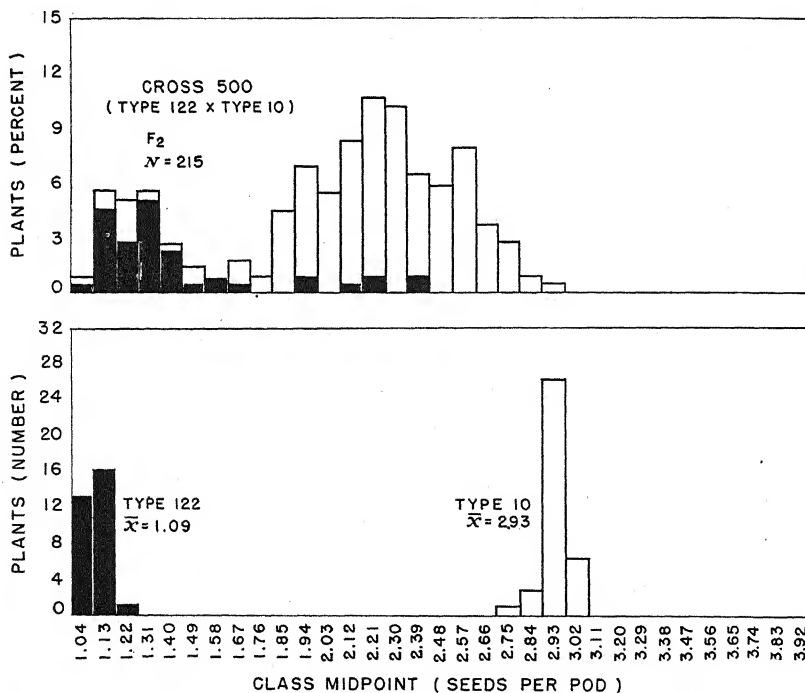
FIGURE 2.—Histograms of parental and F_2 seeds-per-pod distributions of cross 500, typifying those crosses between type 122, which had a low seeds-per-pod mean and oval leaflets, and types with intermediate seeds-per-pod values and normal leaflets. Shaded areas indicate the number of plants within each class which were classified by observation as oval-leaflet type.

The following characteristics were common to most of the F_2 distributions: (1) Certain segregates had values equal to the mean values of both parents; (2) no suggestion of transgressive segregation was apparent; (3) distinct bimodality was exhibited, with the larger portion of the curve comopsed of plants with the higher values; and (4) the modal value of the larger portion of the curve was lower than the mean of the higher parent, and the modal value of the smaller portion of the curve was higher than the mean value of the lower parent.

The bimodality of the F_2 distributions suggested the action of relatively few genes. In all cases the number of segregates in the larger and the smaller portions of the F_2 curve showed a good fit to a 3:1 ratio. The data for the χ^2 tests are shown in table 1.

TABLE 1.—F_2 segregates of 4 intermediate $\times$ low seeds-per-pod crosses, showing segregation in a 3 : 1 ratio of intermediate- to low-value plants. Data for each cross are the numbers of plants in the larger and smaller portions of the bimodal F_2 histograms

Cross	Seeds-per-pod class		χ^2
	Intermediate	Low	
497	90	19	3.33
508	139	39	.91
504	70	18	.97
500	167	48	.82
Total			[1] 6.03

[1]$P = 0.10-0.20$.

INTERMEDIATE $\times$ HIGH

F_2 populations were drived from 12 intermediate $\times$ high crosses. The histograms of crosses 511–523 and 516–524 are shown in figures 3 and 4 typifying the distributions obtained from this type of cross. Seeds-per-pod values of certain F_2 segregates of each cross were equal to the means of both of their respective parents except that in cross 511–523 no F_2 plant was as high in seeds-per-pod value as the mean of the higher parent. The difference between the parents of this cross was the greatest of the 12 crosses, and over 10 percent of the F_2 segregates fell within the range of the higher parent. In all crosses the seeds-per-pod value of the modal class of the F_2 distribution was only slightly higher than the mean of the intermediate parent and was very nearly the same as the mean of the few F_1 plants grown in the field the same season.

The F_2 distributions of three crosses, including 511–523, exhibited a tendency toward bimodality with high values constituting the smaller portion of the curve. These three F_2 distributions showed a good fit to a 3:1 ratio. The data are presented in table 2.

TABLE 2.—*F_2 segregates of the three intermediate × high seeds-per-pod crosses, including cross 511–523 shown in figure 3, which exhibited a tendency toward bimodality. The segregates fit a 3:1 ratio of intermediate to high values*

Cross	Seeds-per-pod class		x^2
	Intermediate	High	
529	146	44	0.03
511–523	150	52	.06
527	49	10	2.04
Total			[1] 2.13

[1] $P = 0.05 - 0.07$.
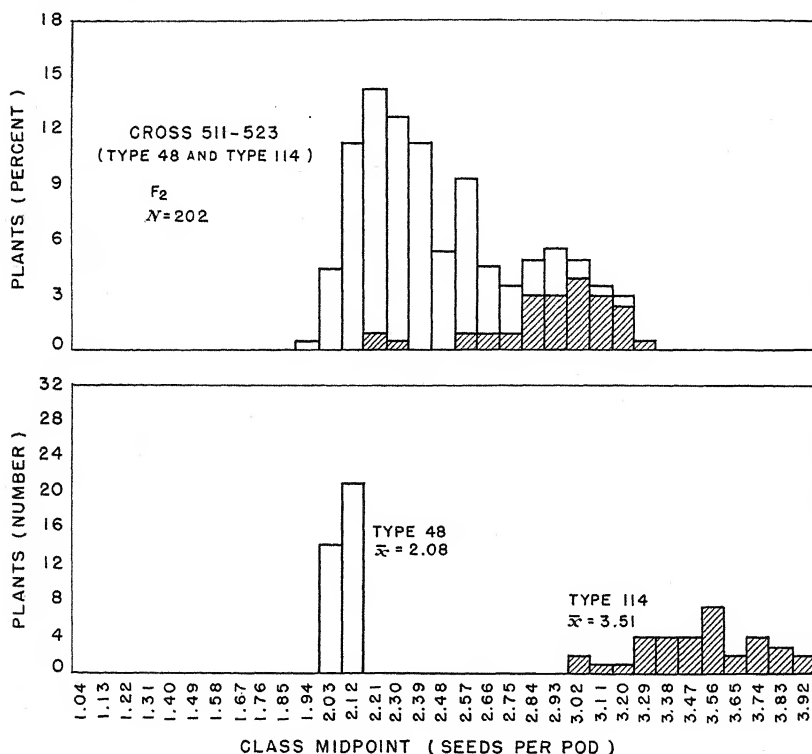
FIGURE 3.—Histograms of parental and F_2 seeds-per-pod distributions of cross 511–523. Cross-hatched areas indicate the number of plants within each class which were classified by observation as narrow-leaflet type.

INTERMEDIATE × INTERMEDIATE

Eight crosses were made between parental types with intermediate seeds-per-pod values. F_2 histograms of two of those crosses are shown in figures 5 and 6. In four of the crosses the F_2 means were intermediate between the parental means; in two crosses the F_2 means approached the means of the higher parents; and in two the F_2 means approached the means of the lower parents. Of the several

crosses studied, cross 159–160 produced the most nearly normal distribution. It is worthy of note that no hint of transgressive segregation was evident in this cross, even with 294 F_2 individuals from parents with quite similar mean values.

Significant positive correlations of 0.65, 0.52, 0.68, and 0.70 were obtained for four crosses when F_2 seeds-per-pod values were correlated with the seeds-per-pod means of their respective F_3 progenies.
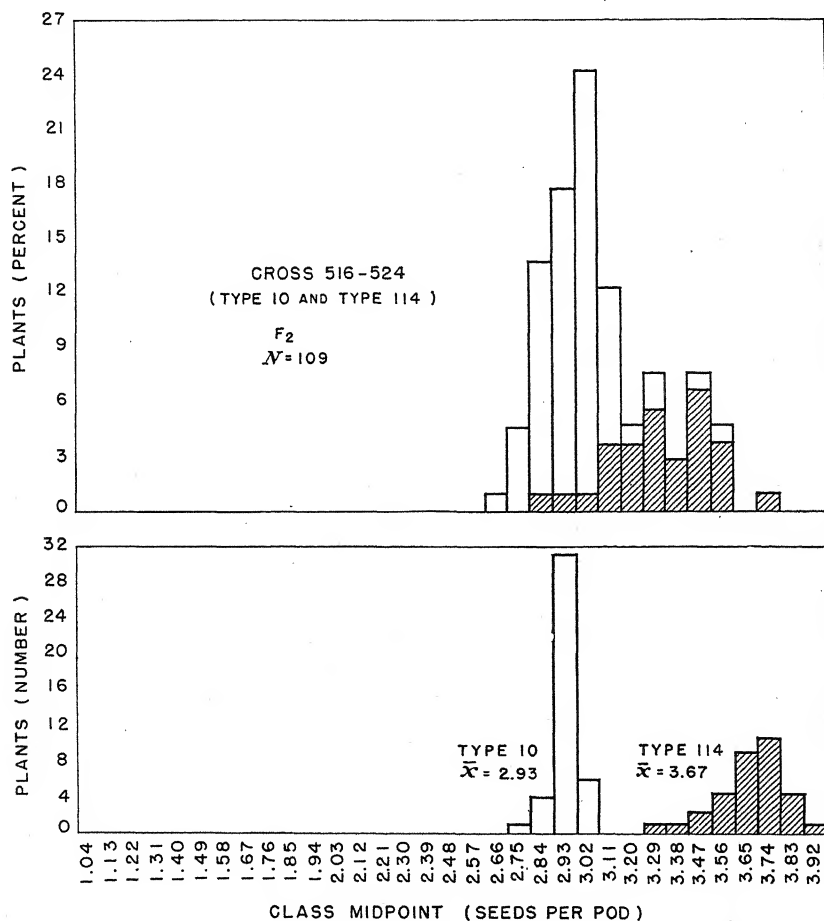
FIGURE 4.—Histograms of parental and F_2 seeds-per-pod distributions of cross 516–524. Cross-hatched areas indicate the number of plants within each class which were classified by observation as narrow-leaflet type.

A negative, but not significant, correlation coefficient of 0.36 was obtained when 22 F_2 seeds-per-pod values of cross 480 were correlated with the coefficients of variability of their respective F_3 progenies. An r value of 0.42 would have been significant at the 5-percent level of probability. A significant negative correlation between F_2 seeds-per-pod values and F_3 coefficients of variability would be considered

evidence that F_2 plants with high seeds-per-pod values were more nearly homozygous for seeds-per-pod genes than those with intermediate values.

LOW × HIGH

The parental and F_2 distributions of cross 522 are shown in figure 7 to represent the two low × high crosses. In both crosses (1) there were no segregates with values equal to the mean of either parent, (2) the value of the modal class of the F_2 distribution was slightly below
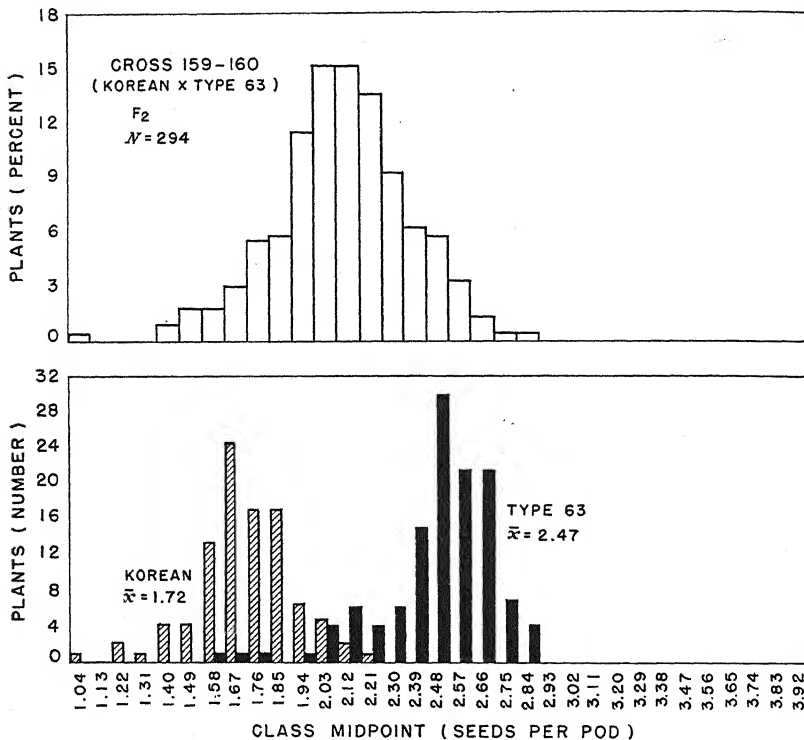
FIGURE 5.—Histograms of parental and F_2 seeds-per-pod distributions of cross 159–160.

the average of the two parents, and (3) both of the F_2 distributions showed a bimodal tendency, with approximately 13 percent of the segregates with the lower value constituting the smaller portion of the curve.

INHERITANCE OF LEAFLET SHAPE

NORMAL × NORMAL

The F_1 and F_2 populations derived from crosses between parents with normal leaflets consisted only of plants with normal leaflets.
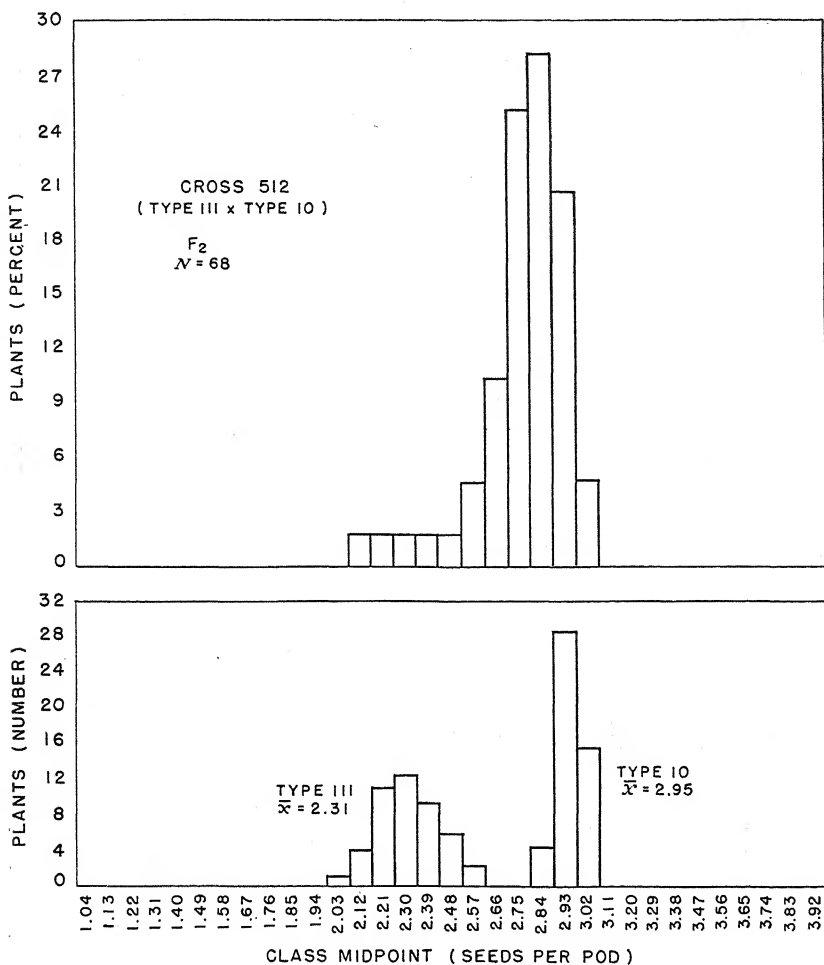
FIGURE 6.—Histograms of parental and F_2 seeds-per-pod distributions of cross 512.

NORMAL × NARROW

From 13 normal × narrow crosses 31 F_2 populations were grown and classified by observation with respect to leaflet shape. Only normal- and narrow-leaflet types were observed in the progenies, although a few types which were classified as normal appeared to approach an intermediate condition. The ratios of normal to narrow plants obtained was, in each case, a good fit to a 3:1 ratio (table 3).
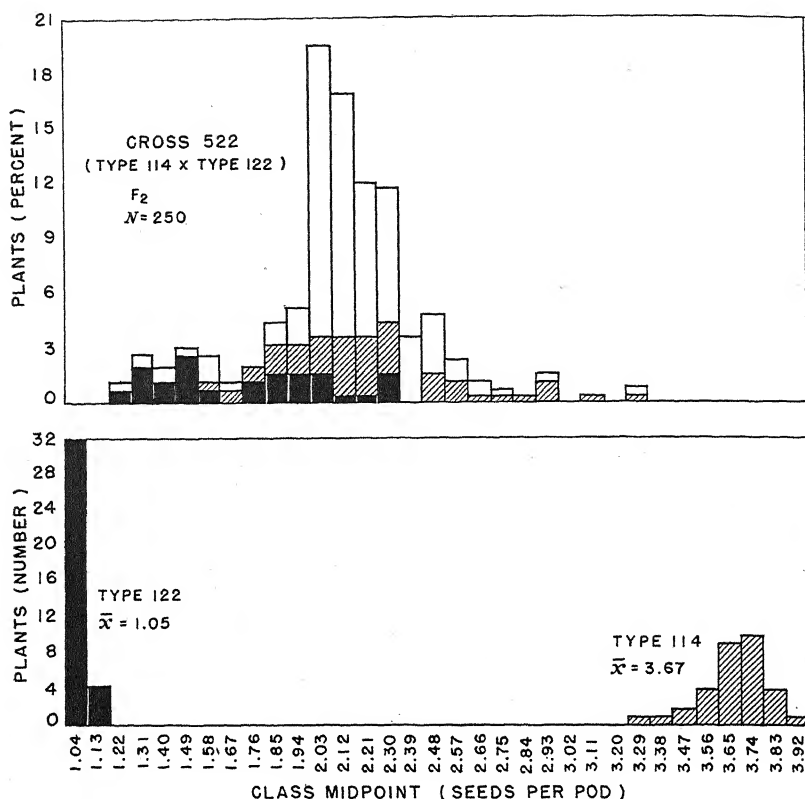
FIGURE 7.—Histograms of parental and F_2 seeds-per-pod distributions of cross 522. Shaded, unshaded, and cross-hatched areas indicate the number of plants within each class which were classified by observation as oval-leaflet, normal-leaflet, and narrow-leaflet types respectively.

TABLE 3.—*F_2 segregates of normal-leaflet $\times$ narrow-leaflet crosses, showing segregation in a 3:1 ratio of normal- to narrow-leaflet plants*

F₁ plant No.	Leaflet shape of F₂ plants		x^2	F₁ plant No.	Leaflet shape of F₂ plants		x^2
	Normal	Narrow			Normal	Narrow	
503A	6	3	0.333	523E	10	1	1.485
503B	8	4	.444	523F	44	9	1.818
503C	40	17	.708	524C	81	20	1.455
503D	8	4	.444	527	42	17	.458
503E	37	9	.725	528	12	4	.000
507A	128	40	.127	516	38	17	1.024
507B	4	2	.222	524A	2	2	1.333
507C	7	1	.667	524B	37	13	.027
507D	9	4	.231	520A	24	7	.097
511A	30	10	.000	520B	15	3	.667
511B	91	21	2.333	529A	14	3	.490
511C	55	17	.074	529B	3	0	1.000
521B	39	11	.240	529C	129	41	.071
523A	56	14	.933	525	42	15	.053
523B	7	1	.667				
523C	3	3	2.000	Total			[1] 20.496
523D	70	20	.370				

[1] $P = 0.90 - 0.95$.

Of the 22 F_2 plants selected at random from cross 480, 6 had narrow leaflets and bred true for narrow leaflets in F_3. Of the 16 with normal leaflets in F_2, 6 bred true for normal in F_3 and 10 segregated in 3:1 ratios of normal to narrow. The 6:10:6 ratio is a good fit to the theoretical 1:2:1.

NORMAL × OVAL

Eight normal × oval crosses produced 23 F_2 populations which segregated for normal and oval. When the ratios were tested against a theoretical ratio of 3 normal to 1 oval the total χ^2 value exceeded that which corresponds to the 1-percent level of probability (table 4). Of the 21 populations, however, 18 had P values above 5 percent. The P value of the group as a whole was lowered by the large χ^2 values of 5 populations. Those 5 populations were from 2 F_1 plants of cross 508 and 3 F_1 plants of cross 515. The following facts suggest that these are chance deviations: (1) Another F_1 plant of cross 508 produced an F_2 segregation which fit the hypothesis, (2) crosses 515 and 508 were reciprocals of crosses 500 and 498 respectively, the F_2's of which fit the hypothesis, and (3) as regards the leaflet-shape gene, cross 515 is probably the same as cross 655, which fitted the hypothesis, since the pistillate parent of cross 655 was a chlorophyll-deficient mutation from the pistillate parent of cross 515.

TABLE 4.—*F_2 segregates of crosses between normal-leaflet and oval-leaflet parents, and χ^2 values when the segregates were tested to a 3:1 ratio*

F₁ plant No.	Leaflet shape of F₂ plants		χ^2	F₁ plant No.	Leaflet shape of F₂ plants		χ^2
	Normal	Oval			Normal	Oval	
497	86	23	0.884	655A	247	67	2.246
498	47	17	.083	655B	198	60	.419
500A	57	10	3.627	655C	123	34	.936
500B	9	0	3.000				
500C	73	21	.355	Subtotal			[1] 24.271
500D	4	3	1.190				
500E	21	6	.111	508B [2]	145	33	3.963
500F	1	2	2.778	508C [2]	64	7	8.681
500G	6	2	.000	515A [3]	75	11	6.837
502	28	10	.035	515B [3]	94	13	9.424
504A	49	9	2.782	515C [3]	115	18	9.326
504B	8	0	2.667				
504C	17	5	.061	Subtotal			[4] 38.231
504D	39	19	1.862				
508A	84	35	1.235	Total			[4] 62.502

[1] $P=0.10-0.20$.   [3] Reciprocal of cross 500; same as cross 655.
[2] Reciprocal of cross 498.   [4] $P=<0.01$.

NARROW × OVAL

The seven F_1 plants from two narrow × oval crosses had normal leaflets. The F_2 progenies contained plants which had either normal, narrow, or oval leaflets. The data presented in table 5 show χ^2 values obtained when the F_2 ratios were tested against a theoretical ratio of nine normal: three oval: four narrow. The P value lies between 50 and 70 percent.

Table 5.—*F_2 segregates of narrow-leaflet × oval-leaflet crosses showing segregation in a 9:3:4 ratio of normal- to oval- to narrow-leaflet plants*

F_1 plant No.	Leaflet shape of F_2 plants			x^2
	Normal	Oval	Narrow	
501A	41	17	14	1.802
501B	5	1	1	.682
522A	32	8	14	.552
522B	76	25	29	.506
522C	33	6	11	2.240
592D	5	0	0	3.889
522E	8	1	2	1.283
Total				[1] 10.954

[1] $P=0.50-0.70$.

F_3 populations were grown from 12 narrow-leaflet F_2 segregates of a narrow × oval cross. Only narrow-leaflet plants were observed in these populations.

F_3 populations were grown from eight oval-leaflet segregates of the above cross. Three of the eight F_3 populations produced only oval-leaflet plants; the remaining five segregated in a 3:1 ratio of oval to narrow (table 6).

Table 6.—*Segregates of F_3 populations grown from 8 oval-leaflet F_2 plants of a narrow × oval cross, showing no segregation in 3 populations and a 3:1 segregation of oval to narrow in 5 populations*

Identity of F_3 population	Leaflet shape of F_3 plants		x^2 (3:1)	P
	Oval	Narrow		
677A15	21		7.000	0.01
677A16	15		5.000	.02-.05
677A20	15		5.000	.02-.05
677A13	22	5	.605	.30-.50
677A14	16	6	.061	.80-.90
677A17	3	2	.600	.30-.50
677A18	1	1	.667	.30-.50
677A21	18	4	.545	.30-.50

LEAFLET INDICES

The parents which were used in the various leaflet-shape crosses differed markedly in the dimensional relationships of their leaflets as evidenced by their frequency distributions for each index. For an index to be of value in the classification of difficult plants, the F_2 distribution of the index should be completely discontinuous; that is, the index for a particular segregate must fall clearly into one or another portion of the F_2 distribution. Such was not the case when any of the indices were applied to any of these crosses. Some of the F_2 segregates, probably the same ones that caused difficulty in classification, fell between the two well-defined portions of the F_2 distribution. None of the indices used seemed, therefore, to be of value in making leaflet-shape classifications.

LINKAGE OF LEAFLET SHAPE AND SEEDS PER POD

If genes for number of seeds per pod were independent of genes for leaflet shape, it would be expected that each seeds-per-pod class of F_2 populations segregating in a 3:1 ratio for leaflet shape would

consist of normal-leaflet plants and oval- or narrow-leaflet plants in approximately a 3:1 ratio. That such is not the case is shown by histograms in figures 2, 3, and 4.

There was a tendency, shown in figure 2, for oval-leaflet segregates from all crosses involving type 122 (one-seeded type with oval leaflets) to have a low seeds-per-pod value. Such a tendency suggests linkage of the recessive gene for oval leaflet-shape with a recessive major gene for the low seeds-per-pod character, on the assumption that the seeds-per-pod character is governed by the combined action of a few major genes and several modifying genes. An indication of linkage intensities was obtained. All normal-leaflet plants having a low seeds-per-pod value and all oval-leaflet plants having intermediate seeds-per-pod value were considered as cross-over types. By this method cross-over percentages of 4.7, 5.1, 6.1, and 10.3 were obtained for crosses 497, 508, 504, and 500 respectively, or 7.9 ± 0.8 percent when the calculations were based on the totals for the four crosses. The ratios are presented in table 7.

TABLE 7.—*F_2 ratios and cross-over percentages between the loci of a major seeds-per-pod gene and the oval-leaflet gene for 4 crosses between type 122, with a low seeds-per-pod value and oval leaflets, and types with intermediate seeds-per-pod values and normal leaflets.*

Cross No.	Leaflet shape according to seeds-per-pod class				Cross-over percentage and probable error
	Intermediate		Low		
	Normal	Oval	Normal	Oval	
497	85	5	1	18	4.7 ± 1.4
508B	137	2	8	31	5.1 ± 1.2
504 ABC	69	1	5	13	6.1 ± 1.8
500	159	8	12	36	10.3 ± 1.5
Total	450	16	26	98	$7.9 \pm .8$

All of the F_2 distributions from the crosses involving type 114 or type 174 (narrow leaflets and high seeds-per-pod values) showed a tendency for narrow-leaflet segregates to have high seeds-per-pod values, as is shown in figures 3 and 4. Such a tendency suggests linkage of the recessive gene for narrow leaflet shape with a recessive major gene for the high seeds-per-pod character. Since not all of these F_2 distributions produced a bimodal F_2 histogram, an estimate of the linkage intensity is not possible for all of them in the manner described above. However, when the method above was applied to those crosses which show the bimodal tendency (529, 511–523, and 527) cross-over percentages of 4, 13, and 13, respectively, were obtained or 9 percent when applied to the totals for the three crosses.

LINKAGE OF LEAFLET SHAPE AND OTHER QUALITATIVE CHARACTERS

LEAFLET SHAPE AND FLOWER COLOR

Flower color is governed by the allelomorphic genes $W_1 w_1$ for purple and white (2). The F_2 ratios obtained indicate that the flower-color genes are not linked with leaflet-shape genes (table 8).

When its fit to a 9:3:3:1 ratio was determined, a narrow-white ×
normal-purple cross had a *P* value of 0.50–0.70. An oval-white ×
normal-purple cross produced an F_2 ratio with a *P* value of 0.50–0.70.

TABLE 8.—*F_2 and F_3 segregations and their respective χ^2 and P values for several
crosses between parents differing in leaflet shape and other qualitative characters,
showing independence of the genes involved*

Characters	XY	Xy	xY	xy	X²	P
Narrow leaflet (*na*) vs. white flowers (*w₁*)	30	9	10	1	1.627	0.50–0.70
Oval leaflet (*o*) vs. white flowers (*w*)	30	9	15	4	2.138	.50– .70
Narrow leaflet (*na*) vs. gray pubescence (*t*)	26	13	5	5	4.805	.10– .20
	55	22	19	13	6.490	.05– .10
Oval leaflet (*o*) vs. gray pubescence (*t*)	67	18	19	4	2.091	.50– .70
	10	4	3	1	.173	.98– .99
	5	2	2	0	.679	.80– .90
Narrow leaflet (*na*) vs. fasciation (*f*)[1]	9	4	2	1	.667	.80– .90
	14	2	2	0	3.728	.20– .30
	11	5	2	2	1.689	.50– .70
	12	5	5	0	1.758	.50– .70
Narrow leaflet (*na*) vs. yellow seed-coat color (*g*)	66	15	20	0	8.677	.02– .05
Components...{*Nana* segregation					1.455	.20– .30
{*Gg* segregation					5.548	.01– .02
{Linkage					1.673	.10– .20
Oval leaflet (*o*) vs. unrestricted seed-coat color (*i*)	66	18	27	8	1.874	.50– .70
Oval leaflet (*o*) vs. green cotyledon color (*d₁d₂*)[2]	78	6	33	2	1.363	.70– .80

[1] F_3 ratios.
[2] Theoretical independence ratio, 45:3:15:1.

LEAFLET SHAPE AND PUBESCENCE COLOR

The genes *Tt* for tawny and gray determine the pubescence color of
soybeans (*2*). The F_2 ratios (table 8) from four crosses indicate that
the *Tt* locus is not linked with those for leaflet shape.

Two normal-gray × narrow-tawny crosses produced F_2 ratios with
P values of 0.05–0.10 and 0.10–0.20 when tested against a 9:3:3:1
ratio.

An oval-gray × normal-tawny cross produced an F_2 population
with a *P* value of 0.50–0.70, indicating independent inheritance
(table 8).

The 250 segregates of an oval-gray × narrow-tawny cross were
tested against the theoretical independence ratio of 27 normal-tawny:
9 normal-gray: 9 oval-tawny: 3 oval-gray: 12 narrow-tawny: 4 narrow-
gray. The *P* value of 0.20–0.30 indicated independence of the genes
involved.

NARROW LEAFLET AND FASCIATION

The fasciated character is recessive and the gene is designated *f*
(*5*). Six of the F_3 populations of cross 480 segregated for both normal
and narrow leaflet-shape and normal and fasciated stems. That these
two characters are not linked is shown in table 8 by the *P* values for
each of the six populations, ranging from 0.20–0.30 to 0.98–0.99.

NARROW LEAFLET AND SEED-COAT COLOR

The genes *Gg* for green and yellow determine seed-coat color (*8*).
A cross narrow-green × normal-yellow produced an F_2 ratio with a
χ^2 value of 8.677, *P*=0.02–0.05, when tested against a theoretical
independence ratio. When this χ^2 value was separated into its com-

ponent parts (*1*), it was noted that the χ^2 value for the *Gg* segregation was the major component, 5.548.   The χ^2 value for linkage of 1.673 indicated that *g* and *na* were not linked (table 8).

OVAL LEAFLET AND EXTENSION OF SEED-COAT COLOR

The multiple allelomorphic series governing the extension of seed-coat color includes i^i which limits color to the hilum and which is allelomorphic to *i* which permits complete seed-coat coloring (*4*). The *P* value of 0.50–0.70 for the F_2 ratio of a cross segregating for normal and oval leaflets and for $i^i i$ indicates lack of linkage of the genes involved (table 8).

OVAL LEAFLET AND COTYLEDON COLOR

The action of the unlinked genes D_1 and D_2 is duplicate, each producing yellow cotyledons (*10*).   When tested against the theoretical independence ratio of 45:15:3:1, the F_2 (table 8) of a normal-yellow $\times$ oval-green cross has a *P* value of 0.70–0.80, indicating absence of linkage of the genes involved.

DISCUSSION

The F_2 data on crosses between normal- and oval-leaflet strains indicate that these strains differ by a single leaflet-shape gene.   The symbols *O* and *o* are suggested for the genes which produce normal and oval leaflets respectively.

Data through F_3 from narrow $\times$ oval crosses indicate that the genotypes associated with particular phenotypes are *Na-O-* normal, *Na-oo* oval, *nanaO-* and *nanaoo* narrow.   As would be expected if the above hypothesis is correct, all narrow-leaflet F_2 segregates produced only narrow plants in F_3.   Likewise three (*NaNaoo*) of the eight oval-leaflet F_2 plants produced only oval plants in F_3, whereas the remaining five (*Nanaoo*) segregated in a 3:1 ratio of oval to narrow.   Also, the 3:8 ratio is a good fit to the theoretical 1:2 ratio.

The F_2 seeds-per-pod distributions indicate that this character is governed by many genes, all of which do not contribute equally to the expression of the character.   The bimodality evident in the low $\times$ intermediate crosses and the suggested bimodality in some of the intermediate $\times$ high crosses indicate a few major genes for number of seeds per pod.   Also, the F_2 distributions of low $\times$ intermediate crosses indicate the action of modifying genes since the values of the modal classes of the two subcurves of these distributions were higher and lower than the means of the low and the intermediate parents respectively.

It is felt that the agreement with a 3:1 ratio of the F_2 seeds-per-pod distributions from all intermediate $\times$ low crosses justifies the assignment of symbols to the major genes involved.   The symbols *Lo* and *lo* are suggested for this allelomorphic pair.   *LoLo* and *Lolo* produce intermediate values and *lolo* produces low values.

F_2 seeds-per-pod distributions of 3 of the 12 crosses between intermediate and high parents showed a   tendency toward bimodality which is in agreement with Takahashi's (*6*) conclusion that the 2 types of parents differ by a single gene.   Takahashi assigned symbols *Ff*

to the allelomorphic pair. It is not known whether the genes involved in the present study are the same as those in Takahashi's material. Neither his symbols nor new symbols are used here since not all of the crosses between normal and narrow parents exhibited the bimodal F_2 distributions. The nearly significant negative correlation coefficient obtained when F_2 values from cross 480 were correlated with their respective F_3 coefficients of variability suggests that the F_2 plants with the higher values were more nearly homozygous with respect to seeds-per-pod genes than those with lower values. If a single major gene pair is involved, F_2 genotypes *aa* would produce high F_2 values and little variability in F_3, F_2 genotypes *AA* and *Aa* would both produce lower F_2 values than *aa* but in F_3 *AA* would produce little variability and *Aa* would produce much variability. The negative correlation mentioned above might have been greater if it were not for the homozygous low seeds-per-pod genotypes *AA* and their respective slightly variable F_3's.

Data from crosses 527, 529, and 511–523 on linkage intensity between high seeds-per-pod and narrow leaflet indicate approximately 9 percent crossing over as compared with 10 percent obtained by Takahashi (*6*).

The question naturally arises as to whether or not the major seeds-per-pod genes form a multiple allelomorphic series. That is, are the gene for an intermediate number of seeds per pod, which is allelomorphic to the recessive high seeds-per-pod gene, and *Lo* the same gene? The following facts suggest that such is not the case. The genes *o* and *na* are both linked with a gene for intermediate seeds-per-pod value with 8 and 9 percent over respectively. If there is only one gene for an intermediate number of seeds per pod, then *o* and *na* would be linked, with either 1 or 17 percent crossing over, approximately, depending on the order of the loci on the chromosome. The χ^2 test was applied to F_2 ratios from the narrow $\times$ oval crosses, testing their fit to both a theoretical linkage ratio with 17 percent crossing over and the ratio for independent inheritance. The *P* values obtained were 0.05–0.10 for the linkage ratio and 0.50–0.95 for the independence ratio; the *P* value for linkage with 1 percent crossing over would be extremely small. It appears therefore that *na* and *o* are not linked and that the dominant intermediate genes that are allelomorphic to both *lo* and the high seeds-per-pod gene are not one and the same gene.

Of special interest in this study has been the association of each of two variations (possibly mutations) from the normal leaflet shape with different genes for number of seeds per pod. In one linkage group the oval-leaflet gene *o* is closely linked with the major gene *lo* for low number of seeds per pod. Likewise, in another linkage group the narrow-leaflet gene *na* is closely linked with the major gene or genes for high number of seeds per pod. The quantitative character, number of seeds per pod, seems to be governed by several genes which are not equal in their manifestation. It appears that a very few major genes in association with numerous minor genes are responsible for the expression of that character.

SUMMARY

Data on the inheritance of number of seeds per pod and leaflet shape in the soybean were obtained from 26 crosses between parents with mean seeds-per-pod values ranging from 1.05 ± 0.01 to 3.59 ± 0.03 and with either ovate (normal), lanceolate (narrow), or oval terminal leaflets. One parental variety was characterized by oval leaflets and a very low mean seeds-per-pod value. Three varieties had narrow leaflets and very high mean seeds-per-pod values. The remaining parental types had normal leaflets and somewhat intermediate seeds-per-pod means, all of which differed significantly.

The data indicate that number of seeds per pod, while somewhat influenced by environmental conditions, is largely governed by a few major and several minor genes. The symbols Lo and lo are suggested for one allelomorphic pair of major genes. Lo exhibits considerable dominance over lo and produces an intermediate seeds-per-pod value; lo produces a low seeds-per-pod value. Certain of the data suggest the existence of a major recessive gene which produces a high seeds-per-pod value as contrasted with an intermediate value produced by its dominant allelomorph.

Plants with oval-shaped terminal leaflets differ from plants with normal leaflets by one leaflet-shape gene. The symbols O and o are suggested for this allelomorphic pair. OO and Oo produce normal leaflets and oo produces oval leaflets.

Data from the normal-leaflet$\times$narrow-leaflet crosses substantiated earlier reports that a single gene difference is involved. $NaNa$ and $Nana$ produce normal leaflets and $nana$ produces narrow leaflets.

The F_1 plants of crosses between narrow-leaflet plants and oval-leaflet plants had normal leaflets and the F_2 population segregated in a 9 normal: 3 oval: 4 narrow ratio. The genotypes associated with particular phenotypes are $Na-O-$ normal, $Na-oo$ oval, $nanaO-$ and $nanaoo$ narrow. The gene na is therefore epistatic to o.

The locus of the oval-leaflet genes Oo is linked with the locus of the genes $Lolo$ for number of seeds per pod with approximately 8 percent crossing over. The locus of Oo is not linked with the loci of the genes for flower color W_1w_1, pubescence color Tt, cotyledon color d_1d_2, or extension of seed-coat color i and its allelomorphs.

The locus of the narrow-leaflet genes $Nana$ is closely linked with the locus or loci of genes for high number of seeds per pod. It is not linked with the loci of the genes for flower color W_1w_1, pubescence color Tt, fasciation Ff, or seed-coat color Gg.

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A STATISTICAL STUDY OF THE RELATIONS BETWEEN FLAX FIBER NUMBERS AND DIAMETERS AND SIZES OF STEMS [1]

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INTRODUCTION

Relatively few anatomical investigations have been made in the United States to determine the relation between the number and magnitude of fibers in cross sections of the flax stem and the size of the stem. Research in this aspect of the flax problem has been confined chiefly to the continent of Europe and the British Isles.

The object of this study was to obtain a measurement of fibers in flax stems grown in Georgia with a view to making comparisons with similar investigations conducted in other regions.

The investigation has been confined to a determination of the number and diameters of fibers in median cross sections; that is, in sections cut from the middle of the stem at right angles to the length. These stems, selected at random, were of different sizes. The relationships between these fiber characters and stem diameters and median areas have also been determined. No attempt has yet been made to correlate the differences with either environmental or hereditary factors, except in a comparison of two varieties.

Most of the previous studies place the diameter of the stem along with the height in first place in providing an indication of the number and size of fibers per stem. Of these the cross section seems the more important; as height, with the exception of the ends, only adds to what one discovers in the section.

Differences of mineral nutrients, of spacing, or of soil have in most cases been shown to affect the fiber count only indirectly by increasing or decreasing the stem size. The results of a statistical study of cross sections of southern-grown flax should, then, establish a basis for some additional investigations for regional agronomic practices and plant breeding, as well as for linen processing, by demonstrating the types of stems with maximum fiber content. Such knowledge is necessary at this time because of the present interest in the Southeastern States in flax production for both fiber and seed.

REVIEW OF LITERATURE

Davin and Searle (*1*),[2] working with flax in Northern Ireland, obtained a high parent-progeny correlation in flower color, time of flowering, percentage of fiber, length of unbranched part of stem, and possibly number of seeds per capsule. They also found that such

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characters as diameter, area of stem, size of fiber, area of fiber, number of fibers, fibers per square millimeter, number of capsules, and seeds are strongly influenced by environment, and show no significant correlation with those of the parent.

Previous writers have found that the largest array of the most representative fibers is in the median area of the stem. Distal sections show fewer and less developed ones, and sections cut at the crown or below contain only a small number of very coarse fibers. Herzog (3), from an average of 25 stems, found 55 fibers in basal sections, 550 in stems at a height of 25 cm., 530 at 50 cm., and 320 at 75 cm. Tammes (8) found that the number of fibers in cross section does not vary much in a single stem except at the lower and upper parts. For these reasons Tammes, Robinson (6), and Davin and Searle (1) all used the fibers from median sections in distinguishing the fiber differences in flax stems of different varieties or of those grown under different environmental conditions.

The characters found to be influenced largely by environment, that is, size of stems, number of fibers per stem, number of fibers per bundle, number of bundles per stem, number of fibers per unit-section areas, and size of fibers, have been shown to be correlated. Tammes (8) found an increase in number of fibers with increasing diameters of stems up to 2.6 mm., but did not obtain the same proportionate increase in number of fibers in larger stems. Robinson (6) and Davin and Searle (1) also obtained a noticeable increase in the number of fiber cells per section with an increase in stem diameter.

The number of bundles in median cross section has been found by most writers to vary somewhat with the diameter of the stem. Tammes (8) has shown that the number of bundles increases with the thickness of the stems, but is more constant in thick stems than in thin ones. Davis (2, p. 4), on the other hand, says: "The average number of fiber bundles is not materially less in a stem of small diameter . . ." Tammes (8) reported that the number of fibers per bundle in stem cross section varies with different stem diameters and is higher in the thicker stems. Davis (2) states that the fiber bundles are necessarily smaller in fine stems as they are crowded together into a smaller girth of cortex.

The numbers of fibers per square millimeter of median stem area has been shown to vary in inverse proportion to the stem thickness. Davin and Searle (1) show an almost constantly decreasing fiber number per unit area with increasing thickness of stems for the Dutch variety. Small stems of the 0.75-mm.² class have a mean of 412.5 fibers; and the large class, 14.25-mm.², have 87.5 fibers per square millimeter. Robinson's (6) data show that the average of his small stems grown in tap water only, 0.678 mm. in diameter, contained 259 fibers per square millimeter, whereas his larger stems grown in N–2P–K solution averaged 1.176 mm. in diameter and contained only 139 fibers per square millimeter.

That diameters of the fibers increase with increased thickness of the stems has been shown by Tammes (8), who reported a large positive correlation. She found no direct influence of the soil on diameter of the fiber except that when seeds were sown far apart there were larger stems and so larger fibers. Davis (2) and Davin and Searle (1) also found that a thin stand produces larger stems and therefore larger fibers.

MATERIALS AND METHODS

The flax straw for the 420 stems used in most of the experimental work was chiefly from the variety Cirrus, a fiber flax; that used for the comparison of varieties was from strains of Cirrus and Triumph. The flax was grown in Brooks County, Ga. It was sown in the fall and harvested the following June. The soil on which it was grown was chiefly Norfolk sandy loam. A complete fertilizer was used at planting, followed by a top dressing with nitrate of soda in the spring.

The flax plant, although normally self-fertilized, does hybridize to some extent. If that cause of variation is added to the mixing that occurs at harvest and in the preparation for distribution, it may be concluded that the usual commercial variety is both a genetical and mechanical mixture of many diverse homozygous strains as well as heterozygous individuals. The flax stems used in this investigation constitute such a mixture, and therefore it would appear that the variations are in part hereditary and in part purely environmental. The fiber characters of stems of different diameters that are discussed in this paper are such as have previously been found to be influenced chiefly by environment, whereas those of stems of the same diameter are influenced more by hereditary factors and can be the basis for future selections.

The stems were brought into the laboratory when completely mature. At first cross sections were made from median portions embedded in celloidin, but this procedure was found to be too slow, and a more rapid method of hand sectioning was devised. Median stem parts, about 2 inches in length, were boiled in water for about 2 minutes to force out the air and soften the stem. They were then taken out and embedded in paraffin in groups of fives. When the paraffin hardened, the blocks were cut down through the ends of the stems and placed in water for several days. After this soaking, cross sections were readily cut by hand with a safety-razor blade. Sections were then mounted in glycerin. Counting fibers and bundles was accomplished both by direct view through the microscope and by projection on a screen.

EXPERIMENTAL RESULTS

RELATION OF NUMBER OF FIBERS PER MEDIAN SECTION TO STEM DIAMETER

The 420 stems that formed the basis of this study have a mean diameter of 1.63 ± 0.021[3] mm., with a standard deviation of 0.43 ± 0.015 mm. About 89.5 percent of them lie between 1 and 2.19 mm. in diameter (table 1). The unusually large or extremely small classes of stems are not represented.

The number of fibers per stem section ranges from 125 to 1,375, with 85 percent falling between 300 and 799 (table 1). The mean for the group is 538 ± 8.807, with a standard deviation of 180.40 ± 6.242 fibers. This is not an unexpectedly large variation when the wide range of stem sizes is considered.

The correlation between the fiber numbers and the stem diameters (fig. 1) is highly significant, with a coefficient of $+0.719 \pm 0.024$. An r value of 0.128 is at the 1-percent level of significance according to Snedecor's (7) table.

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[3] Standard error.
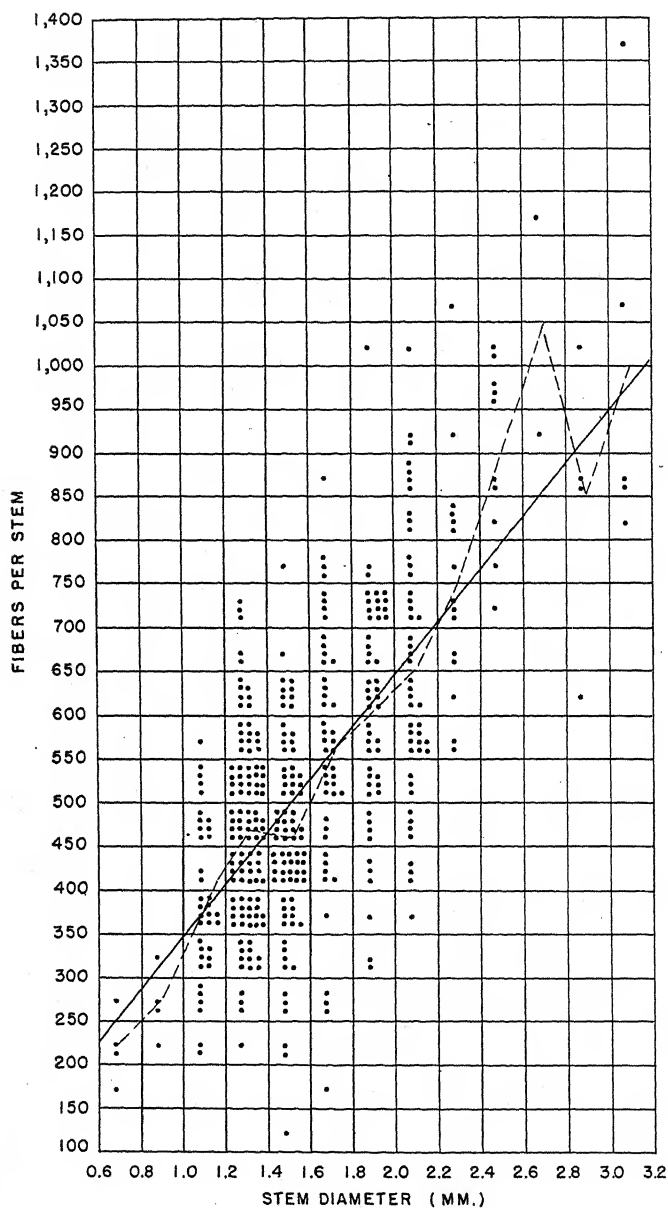
FIGURE 1.—Relation between numbers of fibers per stem and diameters of 420 flax stems.

TABLE 1.—*Proportion of the 420 flax stems studied that fall within each diameter class, fiber-number-per-stem class, fiber-bundles-per-stem class, fibers-per-bundle class, stem-area class, and fibers-per-square millimeter class*

Stem diameters		Fibers per stem		Fiber bundles per stem		Fibers per bundle		Stem areas		Fibers per mm.²	
Range	Proportion within length range	Range	Proportion within specified range	Range	Proportion within specified range	Range	Proportion within specified range	Range	Proportion within specified range	Range	Proportion within specified range
Mm.	*Per-cent*	*Number*	*Per-cent*	*Number*	*Per-cent*	*Number*	*Per-cent*	*Mm.²*	*Per-cent*	*Number*	*Per-cent*
0.6-0.99	1.91	100-199	0.71	10-19.9	3.81	10-14.9	22.38	0-0.9	9.76	0-99	0.71
1.0-1.39	35.24	200-299	5.71	20-24.9	17.38	15-19.9	43.57	1-1.9	46.90	100-199	18.10
1.4-1.79	30.71	300-399	15.48	25-29.9	35.24	20-24.9	23.10	2-2.9	23.33	200-299	40.00
1.8-2.19	23.57	400-499	24.05	30-34.9	28.33	25-29.9	7.62	3-3.9	13.33	300-399	23.57
2.2-2.39	5.95	500-599	23.33	35-39.9	12.62	30-34.9	2.14	4-4.9	4.05	400-499	13.57
2.4-2.79	1.43	600-699	12.86	40-55.0	2.62	35-45.0	1.19	5-5.9	.48	500-599	3.33
2.8-3.20	1.19	700-799	9.29					6-8.5	2.14	600-700	.71
		800-899	4.76								
		900-1,099	3.33								
		1,100-1,400	.48								

The straight line in figure 1 represents a regression of 301.64 ± 14.204 fibers for every increase in stem diameter of 1 mm., or an increase of 60.33 fibers for each 0.2 mm. increase. The error of the estimate is 125.52, and the regression coefficient is highly significant with a *t* value of 21.2, when 2.588 is at the 1-percent level (7).

Figure 1 shows that the prediction line is a very good fit for the *X* means of the *Y* columns up to the 2.3-mm. class of stems, and that the distribution in this area is definitely linear. However, an analysis of variance of the entire distribution shows an *F* value of 2.84 for nonlinearity, when 1.81 is at the 5-percent point of significance. In spite of this it does not seem worth while to plot a curve when 90 percent of the stems fall within an area best fitted by a straight regression line. Then, again, the relatively few large stems would probably cause the calculated values of a curve to be of little value in estimating fiber numbers of other stems of those sizes.

According to the results given above, 51.7 (r^2) percent of the variation in fiber number is accounted for by the variation in stem diameter.

Authors previously referred to have determined that the effect of environment is chiefly to produce larger or smaller stems, and so to increase or decrease the number of fibers. Stems of the same diameter class would then have been grown under approximately the same environment, and the wide variation in fiber numbers in such classes could be attributed to hereditary factors. For example, in stems in the 1.9-mm. class, included in those on which data are given in figure 1, there was one with 1,025 fibers and two with only 325 fibers. This suggests the possibility of isolating superior strains.

None of the authors cited in this paper worked correlation tables between fiber numbers and stem diameters. However, Tammes (8) shows that there is a gradual increase in fibers beginning with stems 0.55 mm. in diameter containing 188 fibers per cross section and continuing up to stems 2.2 mm. in diameter with 1,110 fibers per cross section. The mean diameter of stems studied by her, both large and small, was 1.63 mm. and the mean fiber number was 696. The mean

diameter of stems measured by Tammes was about the same as that of the 420 stems used in the present work, but the mean fiber number was much larger than that of the Georgia-grown stems.

Davin and Searle (*1*) did not calculate the means of stem diameters or of fiber numbers, but in the 1.69-mm. diameter class studied by them the mean fiber number is 681.97. This number is about the same as that found by Tammes for stems of that diameter. The mean of the 1.69-mm. class in the Georgia experiments is about 100 fibers less (fig. 1); however some of the stems in this class contained many more fibers.

RELATION OF NUMBER OF FIBER BUNDLES PER MEDIAN SECTION TO STEM DIAMETER

The sections of the 420 stems used in the study to determine the number of fibers were also employed in a study to determine the number of fiber bundles. In most of the sections the bundles of fibers were quite distinct and were sharply separated by parenchyma cells, but in a few the fibers formed an almost continuous layer.

The number of fiber bundles ranged from 10 to 55, 93.57 percent of the stems containing from 20 to 39.9 bundles (table 1). The mean number of bundles was 29.34 ± 0.277 and the standard deviation was 5.72 ± 0.197 for the mean stem diameter of 1.63 mm.

A significant positive correlation is found between number of bundles and stem diameter, with a coefficient of $+0.396\pm0.042$ (fig. 2) when the 1-percent point is 0.128.
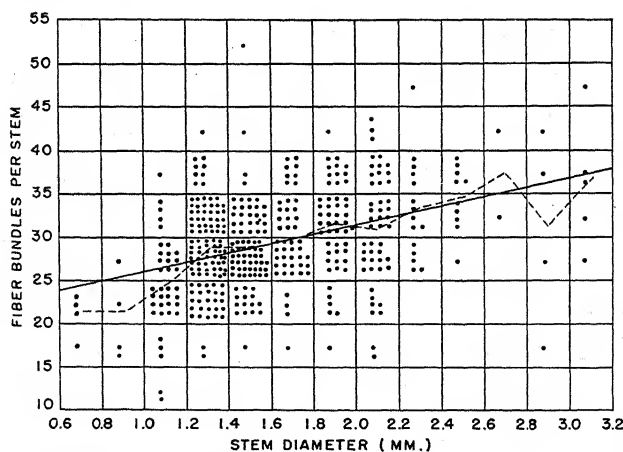
FIGURE 2.—Relation between number of fiber bundles per stem and stem diameter

The number of bundles, shown by the regression line in figure 2, increases 5.32 ± 0.585 for each increase in stem diameter of 1 mm., or an increase of 1.06 for each 0.2 mm. of stem increase. The standard error of the estimate is 5.265, and the *t* value shows a highly significant coefficient of 8.80 when 2.588 is at the 1-percent level.

An analysis of variance to test for nonlinearity shows an *F* value of 1.85 when 1.81 is at the 5-percent point. This distribution constitutes a borderline case, but an inspection of the chart shows that a straight line represents the relationship very well throughout most of the population.

Only 15.68 percent of the variability of the number of bundles per stem can be considered as associated with the differences in stem sizes. The larger stems, accordingly, have a somewhat greater number of bundles than the smaller ones, but this increase is not so marked as in the case of number of fibers.

The range of fiber bundle frequency in stems of the same diameter classes, as shown in figure 2, is almost as great as that of the fibers per stem in figure 1. This variation seems sufficient for isolating lines with selected bundle numbers.

Tammes (*8*) in table 24 of her publication, which gives data on 46 stems of various diameters with a mean of 1.63 mm., shows the mean number of bundles per stem to be 30.37. This number is nearly the same as that found in this investigation.

RELATION OF NUMBER OF FIBERS PER BUNDLE IN MEDIAN SECTION TO STEM DIAMETER

There are two ways in which the number of fibers per bundle may vary. The first type of variation arises from the fact that the bundles in the longitudinal section are elongate-spindle in form, and so apical sections of the bundle will always show few fibers and the maximum number will be in the bundle center. Each stem cross section, therefore, will contain a few extremely small bundles interspersed with larger ones. The second type of variation is found between stems, when the mean number of fibers per bundle in one stem is much smaller or larger than in another. Both types of variation are included in the results of this study.

The average number of fibers per bundle in the 420 stems studied ranged from 10 to 45, 89.04 percent having between 10 and 26 fibers per bundle (table 1). The mean number was 18.87 ± 0.253 with a standard deviation of 5.22 ± 0.181 for the mean stem diameter of 1.63 mm.

There is a highly significant positive correlation between the number of fibers per bundle and the stem diameters (fig. 3). The coefficient is $+0.539 \pm 0.035$. The r value at the 1-percent level is 0.128 as in the previous parts.
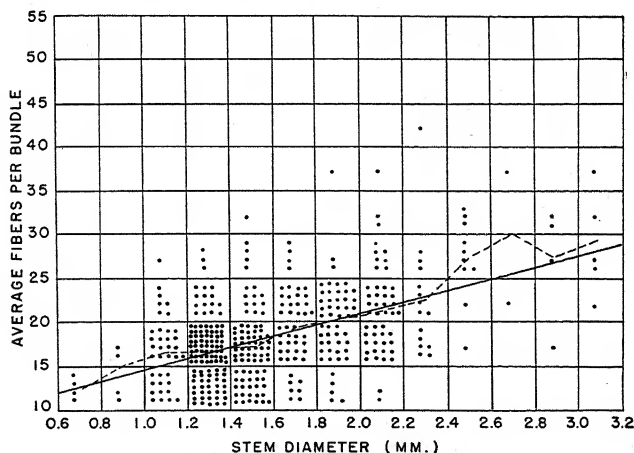
FIGURE 3.—Relation between average number of fibers per bundle and stem diameter.

The straight line in figure 3 shows a regression of 6.54 ± 0.496 fibers per bundle for each increase of 1 mm. in stem diameters; or for each increase of 0.2 mm. in stem diameter there is a corresponding increase of 1.31 fibers per bundle. The error of estimate is 4.41, and the coefficient is highly significant as there is a t value of 13.04 when the 1-percent level of significance is 2.588. The test for non-linearity by analysis of variance shows an F value of 1.404 when 1.81 is at the 5-percent level of significance; hence there is no evidence of nonlinear regression.

As the stem diameters increase the average fiber number per bundle increases, and 29.05 (r^2) percent of the variation in this fiber number increase may be ascribed to increase in stem thickness.

Tammes (8, *table 24*) shows a mean number of fibers per bundle of 22.92 for 46 stems. This figure is somewhat higher than the 18.87 fiber number found in the Georgia stems.

RELATION OF NUMBER OF FIBERS PER SQUARE MILLIMETER IN MEDIAN SECTION TO STEM AREA

Davin and Searle (1) use the term "fiber index" to designate the number of fibers per unit area. The fiber index is obtained by dividing the area of each stem cross section into the number of fibers.

The areas of the 420 stem sections included in this study range from 0.25 to 8.5 mm.², with 83.57 percent between 1 and 3.9 mm.² The mean stem area is 2.14 ± 0.061 mm.², with the standard deviation $1.240.\pm0.043$.
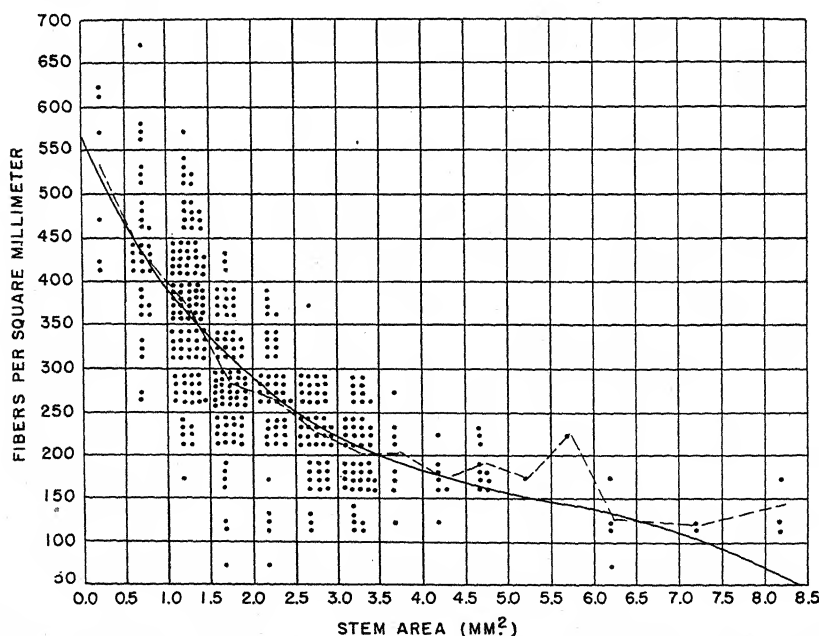
FIGURE 4.—Relation between number of fibers per square millimeter and area of stem in the median cross section.

The number of fibers per square millimeter (table 1) ranges from 50 to 700, 95.24 percent of the stems having between 100 and 499. The mean number of fibers for the 420 stems is 295.70 ± 5.183, with a standard deviation of 106.20 ± 3.674.

A negative correlation was found between the number of fibers per square millimeter and the area of the stem section (fig. 4). The coefficient -0.678 ± 0.027 is highly significant, as the 1-percent level of significance is 0.128.

The regression here is not linear, but fits the curve $X = A + BT_1 + CT_2 + DT_3$, as given by Love (4), except at the lower end. This curve, as shown in figure 4, follows the broken line connecting the X means of the Y columns very closely throughout the blocks of densest population. Table 2 presents in tabular form= what is shown graphically by the curved regression for numbers of fibers per square millimeter for each stem-area class in figure 4. The coefficients are: $A = 215.5$ fibers, $B = 23.5$, $C = 1.71$, and $D = 0.208$. As the stem increases in area the fiber number decreases, but not in uniform proportion.

TABLE 2.—*A comparison of the mean number of fibers per square millimeter in stems of different areas and the predicted values for each stem class*

Classes of stems by areas (sq. mm.)	Means of fiber numbers per square millimeter	Predicted values for curved regression line for fiber numbers per square millimeter	Classes of stems by areas (sq. mm.)	Means of fiber numbers per square millimeter	Predicted values for curved regression line for fiber numbers per square millimeter
0. 25	525	506. 44	4. 75	190	161. 64
. 75	435	431. 24	5. 25	175	150. 72
1. 25	370	368. 16	5. 75	225	140. 72
1. 75	283	315. 98	6. 25	125	130. 36
2. 25	265	273. 44	6. 75	0	118. 42
2. 75	226	239. 29	7. 25	125	103. 64
3. 25	203	212. 28	7. 75	0	84. 76
3. 75	200	191. 16	8. 25	142	60. 56
4. 25	175	174. 70			

In the test of goodness of fit of this curve on the X mean of each stem column there is a χ^2 of 378.84 for the entire curve, indicating a poor fit, but if one stops with the 4.25 stem class χ^2 is only 5.98. The cubic parabola is a good fit for the distribution up to the maximum point of the curve. This curve includes 95 percent of the stems and has a P value, according to Pearson's (5) table, of 0.649438. As was found earlier in the study of the relation of number of fibers per median section to stem diameter, the larger stems do not appear to follow the law governing the smaller ones.

The variability of fiber number per square millimeter associated with the variation in the areas of the stem is 45.97 percent (r^2).

Davin and Searle (1, *table XVI*) have reported for small stems of the Dutch variety, 0.5 to 1 mm.² in area, 412.5 fibers per square millimeter; and for large stems, 7 to 7.5 mm.² in area, 128 fibers per square millimeter. The Georgia-grown stems of equal area have means of 435 fibers per square millimeter for the small stems and 125 fibers for the large ones. This bears out the tendency referred to above for fiber index to decrease with increasing stem size.

These results show that the medium size stems, or even the smaller ones, of equal height have fewer fibers per stem but more per unit area of cross section than larger ones. In equal volumes of small and large stems the small stems would produce the greater number of fibers.

RELATION OF FIBER DIAMETER TO SIZE OF STEMS

The diameters of 50 consecutive fibers in each cross section of the ten smallest stems of the 420 were found and compared with similar measurements of 50 consecutive fibers in each cross section of the 10 largest stems. The difference between the means of the two groups of stems, as shown in table 3, is 31.30 times its standard error. This

TABLE 3.—*Comparison of the diameters of the smallest and largest stems*

Stem class	Range in diameter stems	Mean stem diameter	Standard deviation
	Mm.	*Mm.*	
10 smallest stems	0.65–1.05	0.84±0.039	0.12±0.027
10 largest stems	2.70–3.30	3.00±0.059	0.19±0.041
Difference of means		2.16±0.069	

is more significant than the mean fiber diameter difference given in table 4, which is 3.97 times its standard error. These data indicate

TABLE 4.—*Comparison of the diameters of the fibers of the small and large stems in table 3*

Stem class	Range in diameter of fibers	Mean fiber diameter	Standard deviation
	μ	μ	
10 smallest stems	7.2–28.8	17.32±1.466	4.64±1.036
10 largest stems	7.2–72.0	30.98±3.110	9.83±2.199
Difference of means		13.66±3.438	

that as the stem increases in thickness the diameter of the fiber increases, but to a less degree. Large stems in this group would be expected to have coarse fibers not so well adapted for manufacture of the higher grade products.

The relation between stem sizes and fiber diameters of Georgia-grown flax is approximately the same as that of the Netherlands-grown flax reported by Tammes (8). Here table 32 shows a mean stem diameter of 0.87 mm. for 14 small stems, which have a mean fiber diameter of about 17μ. The four large stems, with a mean-stem diameter of 3.08 mm., have a mean-fiber diameter of 37.18μ.

COMPARISON OF FIBERS PER SQUARE MILLIMETER IN MEDIAN SECTIONS OF THE FIBER FLAX VARIETIES CIRRUS AND TRIUMPH

Median cross sections of 90 stems of Triumph and 90 stems of Cirrus flax grown in Brooks County, Ga., were made for this study. The sections of Cirrus ranged in area from 0.25 to 4.75 mm.², with a mean of 1.81±0.085 and a standard deviation of 0.81±0.061. The number of fibers per square millimeter for each stem ranged from 75 to 625, with a mean of 310±10.468 and a standard deviation of 99.25±7.401.

In this series of sections there is a negative correlation of −0.716±0.052 between the number of fibers per square millimeter and the stem area. This coefficient is significant, because the 1-percent level of significance of *r* is 0.267. This is only a slightly larger negative correlation than that found for the 420 stems.
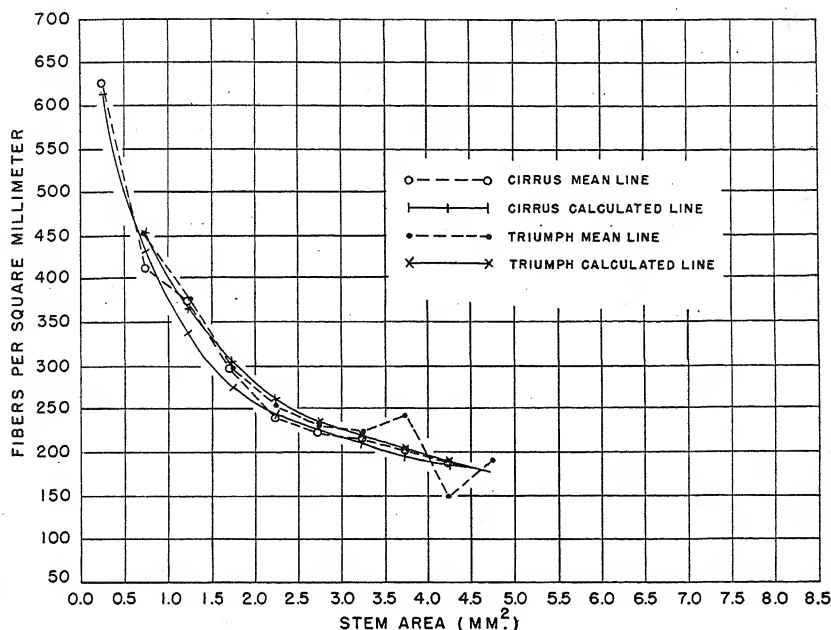
FIGURE 5.—Comparison of the number of fibers per square millimeter and the median-stem areas of Cirrus with those of Triumph flax.

The means of the fibers per square millimeter for each stem-class row for both Cirrus and Triumph are plotted in figure 5 and are connected with broken lines. The predicted values for both varieties are connected with solid lines, and are produced by the same formula used in the study of the relation of fibers per square millimeter to stem area. The one unusually large stem in the 4.75-mm.[2] class found in this random sample of Cirrus is not considered in the curve, as a calculated value based on one stem would have little significance. The curve values for A, B, C, and D are 337.8, 61.7, 15.0, and 2.4, respectively.

The test of goodness of fit of the curve on the X means of the Y columns shows a χ^2 of 6.681 with a P value in Pearson's (5) table of 0.353494.

Within these Cirrus stems 51.3 percent of the variability of the fiber number per square millimeter is associated with the variation in the median stem areas.

The stems in the Triumph variety ranged from 0.5 to 7.5 mm.[2] in area, with a mean of 2.27 ± 0.129mm.[2] and a standard deviation of 1.22 ± 0.090.

The fibers per square millimeter ranged from 125 to 450, with a mean of 292.20 ± 10.107 and a standard deviation of 95.83 ± 7.128. There is also a negative correlation between the number of fibers per square millimeter and the stem areas. The coefficient -0.700 ± 0.053 is significant, as the r value at the 1-percent level of significance is 0.267, as with Cirrus.

The Triumph curve is based on the values of A, B, C, and D, which are respectively 269.6, 31.1, 4.8, and 8.4.

The goodness of fit for this curve shows a χ^2 value of 17.822 for the total, which indicates that the fit is not highly significant. Here, again, the cubic parabola fits the first six classes with 90.91 percent of the population very well, with a χ^2 of 0.768 having a P value of 0.962566 in Pearson's (*5*) table. As was true in the other studies involving number of fibers per square millimeter, the larger stems in these random samples are too few to be expected to follow a law governing the mass of the population.

In the Triumph variety 49 percent of the variability in fiber number per square millimeter is associated with the variation in the stem areas.

The Triumph stems with a mean of 2.27 mm.² were larger than those of Cirrus with a mean of 1.81 mm.² There was also more variation in the stem sizes of the Triumph (table 5). The difference between the means is 3.01 times its standard error, which is significant.

TABLE 5.—*A comparison of the median areas of 90 stems of the Cirrus variety with 90 of the Triumph variety*

Variety	Range in stem areas	Mean stem area	Standard deviation
	Mm.²	*Mm.²*	
Cirrus	0.25–4.75	1.81±0.085	0.81±0.059
Triumph	0.50–7.50	2.27±0.127	1.22±0.090
Difference of means		0.46±0.153	

TABLE 6.—*A comparison of the number of fibers per square millimeter of the median areas of 90 stems of the Cirrus variety with 90 of the Triumph variety*

Variety	Range in number of fibers per mm.²	Mean number of fibers per mm.²	Standard deviation
Cirrus	75–625	310.0±10.467	99.25±7.416
Triumph	125–575	292.2±10.107	95.83±7.143
Difference of means		17.8±14.550	

The mean number of fibers per square millimeter is greater in the Cirrus variety, 310, than in the Triumph with 292.2. This difference is due to the fact that Triumph has larger stems than Cirrus. However, table 6 shows that the difference between the means of the number of fibers per square millimeter is only 1.22 times its standard error, which is not significant.

The correlation between fibers per square millimeter and stem areas is nearly the same, −0.700 for Triumph and −0.716 for Cirrus.

The mean lines in figure 5 show that between the 0.75-mm.² and the 3.25-mm.² classes the mean fiber numbers for the two varieties are almost the same. Cirrus and Triumph stems of equal size, therefore have approximately the same number of fibers per square millimeter of median section. There are, however, other material differences, such as the greater amount of top branching and formation of seed, in the Triumph.

SUMMARY

This study of the relation of flax fiber numbers and diameters to stem sizes in Georgia-grown flax has been made with median or midsections cut from stems selected at random.

The fibers in the median portion of the stem are both more uniform and more numerous than those at the top or bottom.

Cross sections of 420 stems show that as the stem increases in diameter the number of fibers, the number of bundles, and the number of fibers per bundle increase. The correlation is greatest between fiber number and stem thickness. The exceptionally large stems do not exhibit the same degree of linearity of regression as those of medium or smaller diameter.

A negative correlation was found between the number of fibers per square millimeter and the cross-section area of the stem. Large stems have fewer fibers per unit of area than small stems. In this case the regression is nonlinear and again the calculated values for the few very large stems are not worth as much for prediction purposes as those of the rest of the population.

Large stems have coarser fibers than small stems. Diameters of fibers in the 10 largest stems are nearly twice those of the 10 smallest.

A comparison of Cirrus with Triumph, two fiber varieties, shows that the fiber number per unit area of cross section is practically the same in stems of equal size, but as the average diameter of Triumph is larger, the fibers per square millimeter are fewer.

A comparison of fiber numbers per stem section of equal diameter of Georgia flax with published results from the Netherlands and Northern Ireland shows a reduction of about 20 percent in the former.

A study of the correlation charts in this paper shows that within flax stems of equal diameter the range of fiber and bundle numbers is probably wide enough to provide a basis for single-plant selections.

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A METHOD FOR DETERMINING THE RELATIVE COLD HARDINESS OF DORMANT PEACH FRUIT BUDS [1]

By E. M. MEADER, formerly *junior pomologist* [2]; O. W. DAVIDSON, *assistant professor of pomology;* and M. A. BLAKE, *chief in horticulture, New Jersey Agricultural Experiment Station*

INTRODUCTION

In the more northern States where peaches are grown commercially, the fruit buds are frequently damaged by winter weather. In 3 years of the past decade there has been considerable injury to peach buds in commercial orchards in New Jersey, especially to those of the relatively tender varieties. Exceptionally cold winters are sometimes referred to by pomologists and plant breeders as "test" winters, since the relative hardiness of different varieties of fruit can be determined by observing the amount of injury sustained by each as a result of the low temperatures.

One of the limitations to an extensive breeding program to originate hardier peach varieties for northern districts has been the inability to evaluate the cold hardiness of seedlings and varieties without their experiencing a "test" winter. The evaluation of fruit-bud hardiness for a variety of peach is a long and uncertain task, as measured by uncontrollable outdoor conditions. Trees of a variety to be tested must be propagated, grown to fruiting age, and then subjected to critical low temperatures before hardiness determination is possible. A reliable artificial method for testing the cold hardiness of peach fruit buds should therefore be of great value to peach breeders. By the use of such a method trees of newly introduced varieties successful in other peach districts and promising seedlings from the breeding work might be rated for hardiness as grown under orchard conditions in New Jersey and elsewhere before any "test" winter occurs. A practical freezing test for determining the relative cold hardiness of the fruit buds of different varieties of peaches is described herein.

REVIEW OF LITERATURE

Many investigators have studied winter hardiness in plants, and numerous methods have been proposed for measuring the resistance

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of plants to cold. Excellent bibliographies, including many references to orchard observations on the hardiness of fruit trees, have been compiled by Chandler (*8*),[3] Hildreth (*17*), and Potter (*27*). For convenience the methods have been grouped as follows: (1) Indirect determinations that may be correlated with hardiness; (2) direct determinations wherein plants or plant parts are frozen under controlled conditions.

INDIRECT DETERMINATIONS

Beach and Allen (*3*) investigated structure and composition as related to hardiness in the apple. Johnston (*19*) and Strausbaugh (*32*) reported that the moisture content of the buds was directly related to hardiness in the peach and plum. Bakke et al. (*2*), Dunn and Bakke (*15*), and Dunn (*14*) studied relationships of freezing-point lowering of the cell sap, moisture content, ash determination, and a dye adsorption test to hardiness in the apple.

To rate hardiness in alfalfa and in apple, Dexter (*11*), Swingle (*34*), and Stuart (*33*) made use of electrical conductivity measurements of the diffused electrolytes from tissues frozen artificially. Stark (*30, 31*) reported that a hardy apple variety retained more unfrozen water at low temperatures than did a tender variety. Tysdal (*35*) developed a method for studying the relationship of varietal cold hardiness in alfalfa to enzymatic activity. Levitt and Scarth (*20, 21*), in studies on woody plants, used a plasmolytic method for determining osmotic pressures in living cells and suggested the use of a permeability test for predicting cold hardiness.

DIRECT DETERMINATIONS

Chandler (*8*), Allen (*1*), Mix (*23*), Carrick (*7*), Howard (*18*), and Potter (*25, 26*), using an ice-salt mixture to cool insulated chambers to the desired low temperatures, found greater injury to woody tissues (apple, peach, and cherry) by rapid temperature fall than by slow freezing. Hildreth (*17*) designed an apparatus cooled by coils operated by a mechanical ammonia compressor and found that apple twigs exposed to −20° for 12 hours were more severely injured than those held at the same temperature for 3 hours. Magness (*22*) paraffined the cut ends of pieces of roots, placed the roots in glycerin and water, and froze them by gradually lowering the temperature of the solution to certain low points. Dunn (*13, 14*) sealed apple twigs in glass tubes, placed the tubes in an ether bath, and froze the twigs by dropping pieces of solid carbon dioxide in the ether a little at a time. Peltier and Tysdal (*24*), Brierley and Angelo (*6*), and Hansing et al. (*16*) tested the hardiness of alfalfa, grape, and perennial sedums, respectively, by growing plants in containers that could be moved into controlled low-temperature chambers. Treated plants were put in the greenhouse to observe recovery. Cullinan and Weinberger (*10*) exposed peach fruit buds in a chamber set for the desired minimum temperatures, but experienced difficulty in getting their material to the same low point at an unaltered rate of fall on successive dates. Their results indicated that Elberta increased in hardiness during

[3] Italic numbers in parentheses refer to Literature Cited, p. 300.

November, changed little during December, and reached its maximum cold resistance on January 21, 1933, at Beltsville, Md.; Greensboro and Carman were consistently hardier than Elberta. They concluded that varietal differences in hardiness, seasonal growing conditions, size of crop, the stage of development of buds in early and late winter, and quantitative and qualitative differences in shoot growth were factors affecting the survival of buds during periods of critically low temperatures. Smith and Potter (*28*) described an improved freezing chamber for automatically controlling temperatures. Winklepleck and McClintock (*36*) rated the amount of injury to *Prunus* stocks that were exposed to −15° ±1° F. in an insulated box for various time intervals, but no consideration of the rate of fall to the minimum temperature was given.

CHOICE OF METHOD FOR HARDINESS DETERMINATIONS

Of the several methods used by other workers, the diffusion of electrolytes method seemed to offer the greatest promise for rating hardiness of the peach, since it had been so favorably reported for other kinds of plants. A comparison of data obtained by this method in the present investigations with the published results of Blake (*5*) on hardiness of peach varieties under orchard conditions showed no consistent relation between the results of the two methods of evaluating hardiness. Besides the amount of labor and the time involved in making determinations, a number of uncontrollable factors were found to influence adversely the results by this method.

The objections associated with hardiness tests on peach by means of diffused electrolytes led the authors to seek a rapid direct freezing method for evaluating the cold resistance of peach buds. Preliminary work on this problem was undertaken in March 1940, and a simple, practical method was evolved. The description and use of this method, together with the establishment of its limitations, form the basis of this publication.

An electrically operated ice cream cabinet-type refrigerator (fig. 1) was adapted by means of simple equipment to simulate winter conditions as they occur in nature. The refrigerator was equipped with a thermostat that permitted it to operate at temperatures as low as −30° F. To make possible precise settings of this thermostat, a 12-inch pointer of strip metal was soldered to the dial knob (fig. 1). The end of the pointer, when the knob was turned, followed a semicircular graduated scale inscribed on the cabinet, and produced amplificative readings of the small dial scale. The cabinet had a single compartment 21⅛ by 10⅜ by 14⅞ inches deep with a removable hinged lid that fitted tightly upon a rubber insulating collar at the top edges of the compartment.

A rectangular galvanized sheet-iron tank 16⅞ by 9⅛ by 12¾ inches deep, having 2 wire frames forming 4 divisions for holding test tubes and a central one for a vacuum-operated agitator (fig. 2), was placed within the compartment and allowed to rest on five 1-inch wooden blocks to prevent direct contact with the bottom of the cooling compartment. A bath containing 19 quarts of 50-percent ethanol was used in the tank. Twelve-inch glass tubes with a diameter of 1¼ inches were weighted with No. 8 lead shot so that they floated upright in the
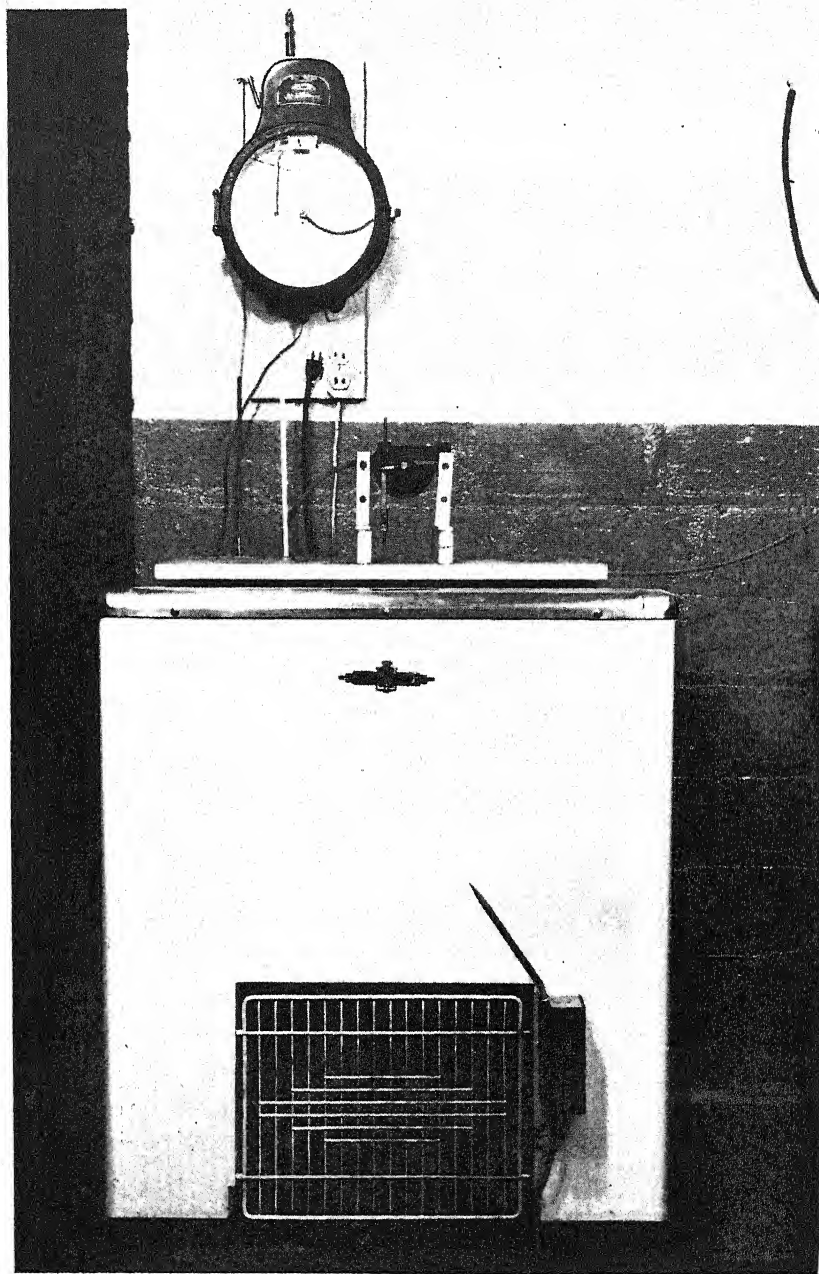
FIGURE 1.—Apparatus used for direct controlled freezing of peach buds.
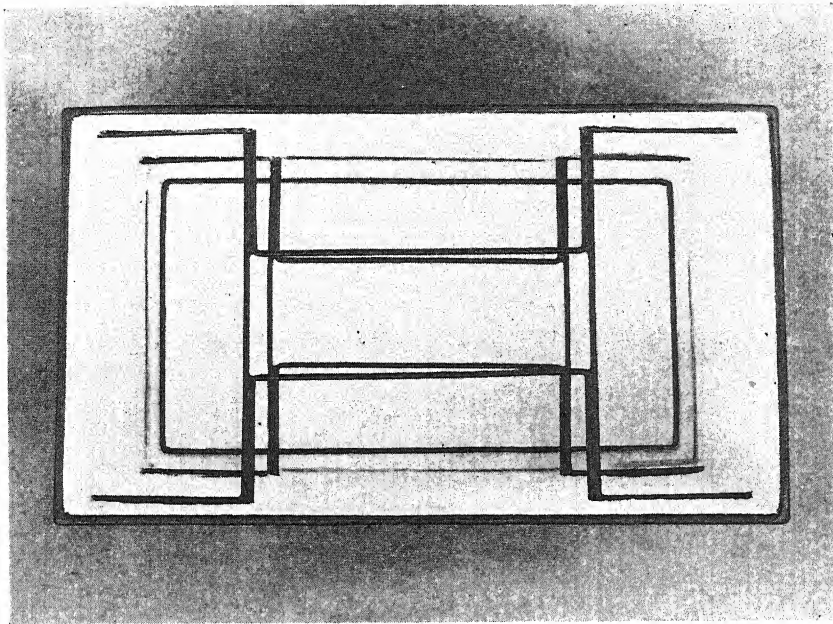
FIGURE 2.—Top view of tank, showing arrangement of compartments in which test tubes containing samples were supported in an alcohol-water bath.
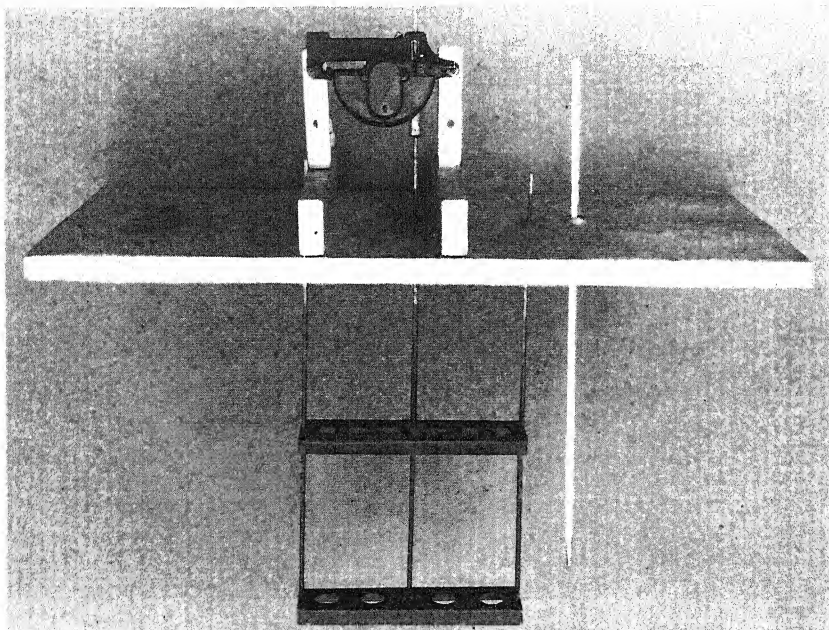
FIGURE 3.—Side view of the agitator that operated in the central compartment of the tank.

bath, which came above the bottom of the rubber stoppers used for sealing samples of twigs in the tubes. Eleven pounds of shot were used in the 50 tubes placed in the tank at one time.

A windshield wiper run by an aspirator pump attached to a nearby water faucet was used to operate the agitator (fig. 3), an arrangement which permitted the maintenance of even temperatures throughout the tank while the bath was cooling to subzero temperatures. The windshield wiper stirrer was mounted on a piece of well-painted ⅞-inch white pine of the proper size to cover the compartment. The agitator operated in a vertical direction (fig. 3) and consisted of two sheet-iron blades, 1⅞ by 7½ inches, mounted one above the other and each provided with six ¾-inch holes.

An accurate thermometer graduated to −32° F. and having a 16-inch stem below this graduation was inserted into the bath through a cork in the cover and adjusted to keep the bulb spaced 2 inches from the bottom of the tank. The bath temperature could be read to 1° and estimated to the nearest 0.1° without removing the thermometer or stopping the agitator.

A continuous record of the temperature changes that occurred in the bath was made by a recording thermometer with a 24-hour chart graded in degrees Fahrenheit (fig. 1, top). Since the remote extension bulb of the recording thermometer was also placed in the bath, the respective readings of the two thermometers could be checked at any time. Any differences were usually within 1° F.

PRINCIPLE FOR AUTOMATIC CONTROL OF RATE OF TEMPERATURE FALL

The antifreeze solution in the tank cooled more slowly than the air in the compartment, and this lag in the temperature change of the antifreeze gave the desired slow rate of cooling. This lag is illustrated in figure 4. Factors that influence the rate of temperature fall of the bath from a given starting temperature and with a given amount of plant material to be frozen are: The specific gravity of the antifreeze solution, its volume, the rate at which the bath is stirred, and the thermostat setting that controls to ±1° F. the minimum temperature of the air in the compartment. In daily runs, when 50 samples were being frozen at a time, the thermostat remained unchanged. Care was taken to put the samples in at the same starting temperature and to maintain the antifreeze at the same specific gravity and volume. When this was done the rate of temperature fall could be duplicated at will, and the total time required to attain the desired low point agreed within 15 minutes for successive runs. As soon as the desired minimum temperature was reached, samples were removed or the cover raised to prevent the bath from dropping to a lower temperature.

For the most part, samples were thawed by removing them directly from the tank to room temperature as soon as the minimum temperature had been reached. In an experiment to test the effects of rapid and slow thawing, the latter was accomplished by raising the cover with 2-inch wooden blocks, one under each end, and forcing air at room temperature (70°−75° F.) into the bath compartment by means of an electric fan while the agitator still operated. This procedure could also be used to raise the bath to the desired starting temperature, but

it was found more satisfactory to remove the tank and place it on a steam radiator in the room because when this was done there was less evaporation of alcohol, and a shorter time was required to reach temperatures above 32°. Samples were allowed to thaw in the sealed tubes until all frost on the twigs had disappeared.
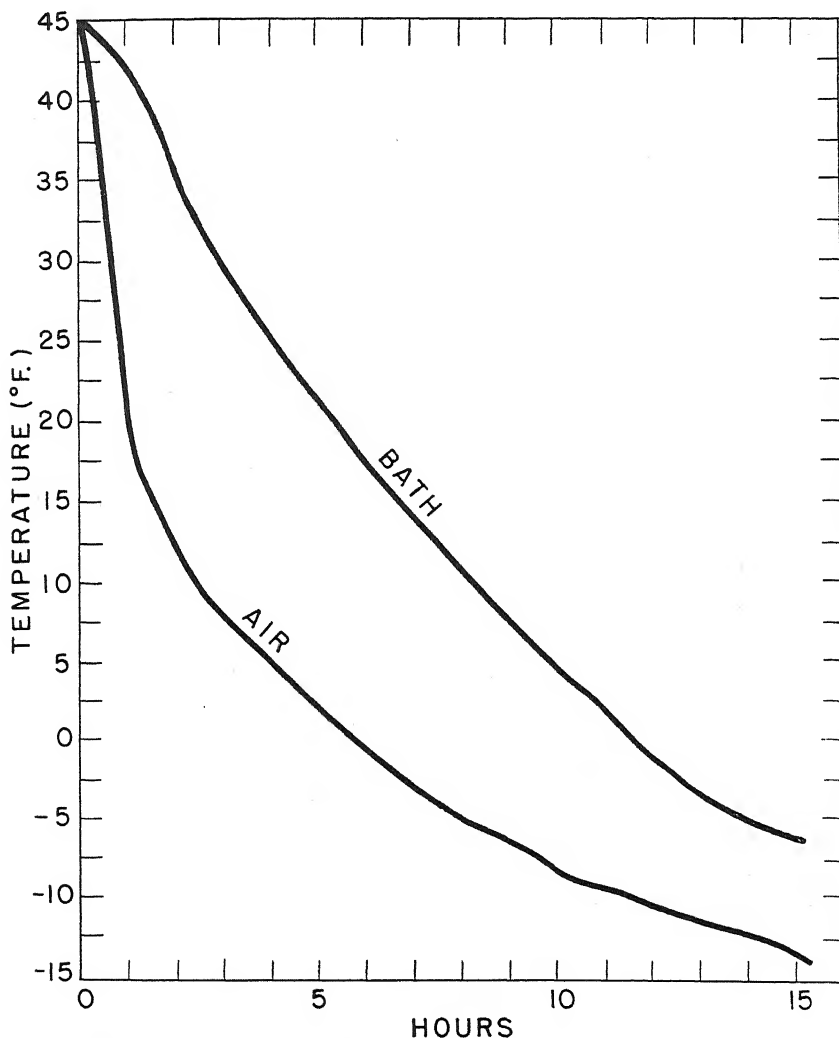
FIGURE 4.—Rates of temperature fall of the bath and the air within the freezer cabinet.

After thawing, twigs were removed from the tubes and allowed to stand in pans of water for at least 24 hours before the buds were examined for injury. After this time interval the fruit buds were cross-sectioned with a razor blade. Injured buds were characterized by a marked brown discoloration, and a water-soaked appearance.

PREPARATION OF SAMPLES

In preliminary studies in 1940 and 1941, samples of the varieties listed in table 1 were taken from orchards of the New Jersey Agricultural Experiment Station at New Brunswick. Uninjured and un-branched terminal twigs 12 ± 1 inch in length and with an optimum number (average number or more for a given variety per 12-inch twig) of buds per twig were selected from the periphery of a tree. The bud set was determined according to the New Jersey standard (4). The twigs were cut at either top or bottom to $10\frac{1}{2}$ inches in length, with loss of as few buds as possible. Ten to 15 twigs were then carefully nested together in each glass tube, and the latter sealed with a rubber stopper.

Beginning with experiment 3, all samples were taken from the Adams orchard in Franklin Park, about 8 miles from New Brunswick. Large composite samples of buds were gathered from at least 10 trees of each of the varieties that were used in these studies. The twigs were brought immediately into the laboratory, cut to length, randomized into samples of 10 or 12 twigs, and sealed in tubes ready for freezing.[4] Both 12 ± 1-inch and 18 ± 1-inch twigs were used. The longer twigs were cut at their midpoints to accommodate them to the tubes. Comparable samples were then given certain treatments as noted under each experiment, a description of which follows.

PRESENTATION OF DATA

Several preliminary trials were made to learn the proper thermostat settings to give a desired rate of temperature fall to a particular minimum temperature. Eight peach varieties known to have wide differences in the hardiness of their fruit buds were used to ascertain the rate of cooling to subzero temperatures that would most effectively differentiate the hardiness of these varieties.

EXPERIMENT 1

On March 28, 1940, the starting temperature of the bath was 37° F., and −8.5° was reached in 19 hours. The starting temperature on March 29 was 39°, and the minimum of −7° was reached at the end of 28 hours. A comparison of data for this experiment with those obtained by Blake (5) at New Brunswick, N. J., for the same varieties under orchard conditions following a temperature of −3° on February 13, 1933, is given in table 1. The mean diameters of twigs in hundredths of an inch and their bud set per 12 inches for the varietal samples are also shown.

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[4] Twigs placed in water for several hours either in the laboratory or in cold storage developed more bud injury during the freezing tests than comparable twigs not placed in water.

TABLE 1.—*Data obtained in artificial freezing of dormant peach buds compared with data for buds frozen under orchard conditions; experiment 1*

Variety	Mean diameter (hundredths of an inch) of twigs frozen artificially					Frozen under orchard conditions Feb. 13, 1933 [1]	
	Top	Bottom	Bud set per foot	Total number buds	Percent buds alive	Bud set per foot	Percent alive
	Mar. 28, 1940						
41 S. D.[2]	11.8	15.4	25	182	56	18	65
Dewey	10.3	14.0	15	113	51	12	85
Golden Jubilee	9.9	14.4	22	158	37	18	38
Triogem	10.3	15.1	19	134	28	20	13
Cumberland	10.9	15.1	28	141	21	32	23
Slappey	10.8	14.2	15	138	20	17	30
White Hale	10.8	14.8	13	101	5	16	7
Elberta	10.9	14.6	12	94	3	13	5
	Mar. 29, 1940						
41 S. D.	13.8	17.1	25	145	74	18	65
Dewey	12.8	17.0	15	135	61	12	85
Cumberland	11.7	15.6	28	123	41	32	23
Slappey	11.6	15.5	15	164	41	17	30
Golden Jubilee	10.9	15.2	22	149	38	18	38
Triogem (N. J. 70)	11.4	15.6	19	109	21	20	13
Elberta	12.7	16.8	12	132	5	13	5

[1] These data were reported by Blake (*5*) for samples of 100 buds taken from orchard-growing trees that were subjected to a temperature of −3° F. on Feb. 13, 1933.
[2] Selected seedling from cross of Slappey×Dewey.

EXPERIMENT 2

Freezing tests were resumed in the winter of 1940–41, and on January 2, 50 varieties were frozen from a starting temperature of 42° to −4° F. in 15 hours. On January 3 the starting temperature was 41°, and −4° was reached in 17 hours. The thermostat setting was unchanged, but some water was added to the bath to give the proper volume. This caused a 2-hour delay in freezing, a fact that shows the necessity for checking the specific gravity of the solution if a duplicated rate of freeze is desired. A few of the 50 varieties frozen that have a wide range in relative hardiness are listed in table 2 for comparison with orchard data.

The following experiments designed to ascertain the effects of (1) various rates of freezing; (2) different starting temperatures of the bath; and (3) various critical low points for removal of samples when the rate of fall had been the same, were performed to establish the limitations of technique for the direct freezing method.

The number of live and killed buds on each of the 12- and 18-inch twigs was recorded. Variation in the percentage of live buds on different twigs occurred. Chi-square values were determined according to methods of Snedecor (*29, pp. 164–167*) to see if these variations were within the limits of random sampling from homogeneous material. The data for several twigs were grouped at random for com-

puting chi square in R × 2 tables. Each group contained at least 50 to 100 buds. When only sampling variation from the mean percentage of buds alive for a variety resulting from a given treatment had been proved, differences in the percentages of buds alive in the different treatments were tested by chi square calculated in 2 × 2 tables with a single degree of freedom. Significant differences between treatments are indicated in the tables that follow.

TABLE 2.—*Data obtained in artificial freezing experiments of dormant peach buds compared with data for natural freezing injury in orchard; experiment 2*

Variety	Frozen artificially				Frozen under orchard conditions [2]
	Jan. 2, 1941		Jan. 3, 1941		Feb. 13, 1933
	Total buds	Buds alive	Total buds	Buds alive	Buds alive
	Number	*Percent*	*Number*	*Percent*	*Percent*
Marquette No. 1 [1]	174	77.5	193	62.6	
Marquette No. 2			178	68.5	
41 S. D. No. 1	148	64.2			65
41 S. D. No. 2			175	51.4	
Duke of York			122	55.7	54
Early Rose	239	60.7	188	51.1	
Greensboro	246	53.3	175	46.6	90
Veteran	105	54.3			
Golden Jubilee			180	32.2	38
Garden State [3]	107	21.5	123	17.9	
Cumberland	249	20.9	246	22.0	23
Hiley	216	19.9	181	9.4	5
Elberta			98	14.3	5

[1] Trees 1 and 2 sampled.
[2] Data by Blake (*5*); 100 selected buds of each variety were examined.
[3] Nectarine.

EXPERIMENT 3

The effects of rate of temperature fall are closely associated with the starting temperature, the following treatments being used: (1) Samples put in bath at 38° F. and cooled to −6° in 13½ hours; (2) Samples put in bath when it had dropped to 20° and cooled to −6° in 9½ hours; (3) samples put in at 10° and lowered to −6° in 6½ hours; and (4) samples put in at 0° and reached the minimum of −6° in 3 hours. Samples in treatments 2, 3, and 4 were kept at 45° until put in the bath when this had reached the temperatures indicated. All samples of the four treatments reached the minimum of −6° simultaneously and were thawed similarly, a return to 39° being accomplished in 6 hours; that is, the treatments varied only in the time and temperature at which they started. To maintain the level of the bath just above the bottom of the stoppers in the tubes and yet not change its volume when tubes of different treatments were removed from or put into the tank, bottles weighted with dry sand were used to displace the solution in amounts equal to the volume of the tubes (usually 12 per treatment) included in the treatments. The thermometer was carefully watched to see when the bath started to cool again after the samples of each treatment (at approximately 45°) were added. This occurred in less than one-half hour in each case. Data obtained in experiment 3 are presented in table 3.

TABLE 3.—*Data on effects of rate of temperature fall and starting temperatures on survival of dormant peach fruit buds of 2 varieties, Feb. 3, 1941; experiment 3* [1]

Treatment			Summercrest		Cumberland	
No.	Starting temperature of bath	Time elapsed before temperature dropped to −6° F.	Total buds	Buds alive	Total buds	Buds alive
	° F.	Hours	Number	Percent	Number	Percent
1	38	13½	344	61.6	405	53.1
2	20	9½	378	50.0	428	35.3
3	10	6½	410	2.2	457	2.8
4	0	3	366	0	452	0

[1] *P* value for chi square less than 5 percent where percentage difference in treatment comparison was as much as 8.5, and less than 1 percent where percentage difference in treatment comparison was as much as 11.6.

EXPERIMENT 4

The purpose of experiment 4 was to determine whether measurable differences in injury to fruit buds would occur if buds were taken from the cool temperatures of the orchard to a warm room and, after being prepared there, frozen to the desired low points. If differences did occur, the alternative would be to prepare the samples at the temperature prevailing in the orchard and then have the bath at the same temperature before putting in the samples. The effect of putting in samples at 20° and 10° F. for starting temperatures of the bath and freezing them to −6°, but at a slower rate than in experiment 3, was also tested.

TABLE 4.—*Data on the effect of certain starting temperatures and a relatively slow rate of freeze on survival of dormant fruit buds of 4 varieties of peaches, Feb. 10, 1941; experiment 4* [1]

Treatment				Summercrest		Cumberland		Triogem		Golden Jubilee	
No.	Initial temperature of buds	Starting temperature of bath	Time elapsed before temperature dropped to −6° F.	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive
	° F.	° F.	Hours	Number	Percent	Number	Percent	Number	Percent	Number	Percent
1	45	40	21¾	439	75.4	767	76.3	512	78.9	819	85.2
2	83	40	21¾	496	81.7	661	72.0	497	83.1	614	85.8
3	45	20	14¼	455	63.1	609	50.9	530	68.9	715	47.1
4	45	10	8¾	509	7.5	674	7.0	515	8.2	661	2.6

[1] *P* value for chi square less than 5 percent where percentage difference in treatment comparisons was as much as 6.3; and less than 1 percent where percentage difference in treatment comparisons was as much as 14.2.

After being sealed in the tubes, all samples were kept at 45° F. until they were treated as follows: (1) put in the bath with a starting temperature of 40° F. and frozen to −6° in 21¾ hours total time; (2)

samples brought to 83°, as determined by a thermometer inserted through a stopper into the center of the twigs in one tube, and then put in the bath simultaneously with treatment 1; (3) samples put in the bath when it had dropped to 20° and lowered to –6° in 14¼ hours; (4) samples put in the bath at 10° and lowered to –6° in 8¾ hours. When the minimum of –6° was reached, the temperature of the bath was brought up to 28° before all samples were removed to room temperature. Relevant data are given in table 4.

EXPERIMENT 5

To ascertain the most practical starting temperature of the bath, samples of the same four varieties that were used in experiment 4 were prepared and kept at 45° F. until given the treatments indicated in table 5.

These treatments, as in the previous experiments, provided for successively greater and faster drops from the initial temperature (45°) to the bath starting temperature with decreasing time intervals to the minimum temperature of $-6°$. All samples remained in the bath until it had returned to 36° in 3¼ hours. The summarized data appear in table 5. For the first time after chi square tests for homogeneity of buds on the pooled 12-inch and 18-inch twigs had been made, it was found necessary to separate the two lengths in order to have uniformity within samples of buds that had been treated similarly throughout the experiment. This applied to the Cumberland and Golden Jubilee varieties, which apparently had consistently hardier buds on 18-inch twigs than on 12-inch twigs, but not to Summercrest or to Triogem.

TABLE 5.—*Data on effect of bath starting temperatures as related to survival of peach fruit buds of 4 varieties and the buds on 12-inch and 18-inch twigs when frozen to a minimum of —6° F., Feb. 13, 1941; experiment 5* [1]

Treatment			Summercrest		Cumberland				Triogem		Golden Jubilee			
					12-inch twigs		18-inch twigs				12-inch twigs		18-inch twigs	
No.	Starting temperature of bath	Time elapsed before temperature dropped to –6° F.	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive
		Hours	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1	40	15½	356	62.1	566	48.6	380	52.1	414	66.2	257	49.4	149	68.5
2	30	12¾	321	60.4	269	30.1	204	41.2	402	55.2	239	47.3	199	57.8
3	27.5	12	359	59.3	296	31.4	221	47.5	344	57.0	254	49.2	171	54.4
4	25	11½	310	68.1	343	24.8	221	41.2	349	61.0	229	31.9	217	54.8
5	22.5	10¾	333	45.9	286	17.1	194	42.3	332	53.3	246	26.4	188	39.4
6	20	9¾	311	46.3	283	13.4	184	29.9	383	41.0	239	19.3	222	41.9

[1] *P* value for chi square less than 5 percent where percentage difference in treatment comparisons was as much as 7.7: and less than 1 percent where percentage difference in treatment comparisons was as much as 10.9.

EXPERIMENT 6

The effect on peach buds of a difference of 1° or 2° within the critical range of minimum temperatures at which buds are injured, when the

rate of fall from the same bath starting temperature was similar for all samples, was determined, as shown in table 6. N. J. 66, which is known to have tender fruit buds easily injured by subzero temperatures under orchard conditions, was substituted for Triogem in this and the following experiment. Twelve tubes of each of the four varieties tested were placed in the bath at 30°. Since the tubes had been held at room temperature (70°–75°), they raised the bath to 38° before it started to cool. When subzero temperatures were reached samples were removed from the bath at six low points as shown in table 6. The time interval for the bath to reach each minimum temperature from its starting temperature of 38° is also listed. All samples, after removal from the bath, were allowed to thaw at room temperature in the sealed tubes.

TABLE 6.—*Summary of data on the effect of 6 subzero temperatures on the survival of fruit buds of 4 peach varieties frozen simultaneously at the same rate of temperature fall, Feb. 25, 1941; experiment 6 [1]*

Treatment			Summercrest		Cumberland		N. J. 66		Golden Jubilee	
No.	Minimum temperature	Time elapsed before temperature dropped to low point	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive
	°F.	Hours	Number	Percent	Number	Percent	Number	Percent	Number	Percent
1	−4	13¾	321	82.2	464	71.6	310	41.3	387	86.8
2	−6	15	340	71.5	468	57.9	357	[2] 41.2	347	70.6
3	−7	16¼	330	60.3	498	38.2	317	17.7	380	58.2
4	−7.5	17¼	373	53.6	502	30.7	347	[2] 19.9	369	56.1
5	−8	18	309	46.0	470	30.4	386	16.2	367	49.9
6	−9	19¼	298	37.6	499	21.0	318	16.0	352	41.8

[1] *P* value for chi square less than 5 percent where percentage difference in treatment comparison was as much as 7.5; and less than 1 percent where percentage difference in treatment comparison was as much as 9.4.
[2] *P* value in chi square test for homogeneity less than 5 percent.

EXPERIMENT 7

Experiment 7 was similar in purpose and details to experiment 6, except that the rate of freezing to lower temperatures was relatively much slower. Five progressively lower settings of the thermostat were made to obtain prolonged slow cooling (an average change of approximately 1.25° per hour) to subzero temperatures from the bath starting temperature of 32° F. The six treatments and the data obtained are shown in table 7.

DISCUSSION OF FREEZING METHOD AND DATA OBTAINED BY ITS USE

APPARATUS

No record was found in the literature of the direct use of the lag of a bath of antifreeze solution behind the air temperatures in a cooling chamber for automatically producing the desired rates of temperature fall to subzero temperatures. The apparatus described herein is simple in construction, requires no extra insulation, and with reasonable care

in operation will give continued good service. An important advantage to controlling the temperature of a bath over attempting to maintain air temperatures in a chamber at desired points is that samples may be put into the bath at different times or removed at subzero temperatures without causing appreciable fluctuations in its temperature.

TABLE 7.—*Summary of data on the effect of subzero temperatures on survival of peach fruit buds of 4 varieties frozen simultaneously at the same prolonged slow rate of temperature fall; experiment 7, Feb. 27, 1941* [1]

Treatment			Summercrest		Cumberland		N. J. 66		Golden Jubilee	
No.	Minimum temperature	Time elapsed before temperature dropped to low point	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive
	°F.	Hours	Number	Percent	Number	Percent	Number	Percent	Number	Percent
1	−6	30¼	306	51.6	551	[2] 64.1	370	66.5	368	85.3
2	−8	31¼	362	37.8	550	[2] 52.5	344	48.5	380	80.5
3	−9	32¾	400	27.8	678	35.1	365	38.4	391	64.7
4	−11	34	360	12.8	449	18.0	354	11.0	370	55.9
5	−13	35¾	308	.6	491	4.5	376	1.1	386	7.0
6	−15	37¾	385	0	488	.4	353	.6	359	1.1

[1] *P* value for chi square less than 1 percent where percentage difference between treatments was as much as 8.8.
[2] *P* value in chi square tests for homogeneity less than 1 percent.

It is essential that the antifreeze solution used should give adequate protection at the low temperatures that the operator of the apparatus desires. If ice crystals start to form in the bath, the drop in temperature is retarded for a time because of the liberation of latent heat, and the agitator is run with difficulty. An antifreeze solution of ethyl alcohol and water was chosen because the mixture was readily available and relatively inexpensive, and it had the decided advantage of leaving the tubes clean and dry for handling. Evaporation made necessary the occasional replenishing of the bath with alcohol to give the desired specific gravity for freezing protection at subzero temperatures and to maintain its original volume. A hydrometer of the type commonly used for testing solutions in automobile radiators was found convenient for testing the freezing protection of the bath. To equalize temperatures throughout the tank, the bath was thoroughly stirred at all times while cooling.

DATA TABLES 1 AND 2

From the data presented in tables 1 and 2, it can be seen that when Elberta is so frozen artificially that nearly all its buds are killed (1 to 15 percent alive), the other varieties listed compare favorably with orchard data by Blake (5) for the same varieties at New Brunswick. The differences observed may be due to different degrees of hardiness of trees within the same variety or to the vagaries of sampling. Beginning in January 1941, this method for hardiness determinations of peach fruit buds by direct controlled freezing was put into daily practical use. Fifty samples were prepared and frozen in such a

manner as to leave less than 15 percent of the buds of Elberta alive for comparative purposes. Some hardy varieties, like Marquette, or 41 S. D., were also included in each lot of samples. With these varieties for criteria, the fruit-bud hardiness of varieties or seedlings the hardiness of which had not been tested in cold winters or was unknown could be evaluated. In the case of crosses, samples of parental varieties were frozen along with samples of their progeny. It is necessary to anticipate the type of freeze (rate of temperature fall to a certain low point for removal of samples) that will permit between 1 and 15 percent of the buds of a tender variety like Elberta to remain alive. When this is done, individuals more tender than Elberta can be recognized. If a procedure similar to those of experiments 6 and 7 is used, the proper subzero temperature for removal of samples to give the desired injury to Elberta buds can be determined in one trial freeze. In early winter it may be necessary to use progressively lower minimum temperatures before removal of samples when a particular rate of temperature fall is used, since there is a general increase in the hardiness of peach fruit buds during this period (*10*).

DATA TABLES 3 AND 4

When the rate of cooling from the starting temperature of 45° F. to the same subzero temperature (−6°) increases, the injury to the fruit buds also increases. This fact applies for the two freezes on the two successive dates, the second being relatively slower than the first. In parallel treatments, except for the rate of temperature fall, considerably more live buds are present in the slower freeze. As tested by chi square, significant differences between treatments are indicated in all cases, except treatments 1 and 2 in table 4. Evidently bringing samples to the laboratory, in which they experience temperatures above those prevailing in the orchard, has little effect on the results obtained by this method. Hildreth (*17*) practiced cooling his freezing chamber to the outside orchard temperature before putting samples into the freezer. Chandler (*8*) has shown that rate of thawing has no influence on the amount of injury to peach fruit buds. His findings were substantiated by unreported preliminary work in the present studies.

DATA TABLE 5

For all the treatments shown in table 5, the buds on 18-inch twigs of Cumberland and Golden Jubilee proved consistently hardier than those on 12-inch twigs. Chi square tests for homogeneity showed only variations within the limits of random sampling for the pooled data of 12-inch and 18-inch twigs of Summercrest and Triogem, but uniformity within treatments for Cumberland and Golden Jubilee was attained only by separating the data for the two lengths of twigs. Cullinan (*9*) and Cullinan and Weinberger (*10*) have recognized differences in the hardiness of peach fruit buds on qualitatively different twigs as indicated by length measurements. This fact, supported by data in this study, suggests that selected samples of similar length twigs of different varieties should be used when the buds are to be tested for cold hardiness.

In a comparison of treatments (table 5), Summercrest shows a significant difference between treatments 3 or 4, but the tendency is in

the opposite direction from that expected. A highly significant difference is shown between treatments 4 and 5, and also between treatments 3 and 5. Some unknown disturbing factor may have been responsible for the high value for treatment 4. Summercrest showed significant cold-hardiness differences in samples that were started at 27.5° and 22.5° F. respectively. Cumberland, for both twig lengths, Triogem, and the 18-inch twigs of Golden Jubilee showed significant differences between treatments 4 and 5, i. e., for samples started at 25° and 22.5°, respectively. The effect of starting samples at the several points between 40° and 20° F. was to provide a series of progressively more rapid rates of temperature fall from 45° to the particular starting temperature chosen. Chandler (*8*) froze some buds slowly one-half way down to the killing temperature and rapidly for the rest of the way, others rapidly one-half way and slowly for the rest of the way, and still others slowly all the way to the critical minimum temperature of −16° C. (3.2° F.) used in the tests. The halfway point was −12° C. (10.4° F.). He found that fast freezing in the upper part of the range (above 10.4° F.) gave the greatest injury. Elberta was the only peach variety that he used.

From the present study it is evident that rapid rates of temperature fall well above 10.4° F. can cause appreciable differences in fruit-bud injury when a critical low temperature is reached before the buds are thawed. Data from experiment 5 show the injurious effects of rapid rates of temperature fall somewhere between 30° and 20° for all of the four varieties tested. Varieties apparently react differently to the progressively rapid rates of temperature fall. It is of interest in this connection that Dorsey (*12*) has fixed 27° F. as the critical point for ice formation in peach fruit buds. That the more tender buds react first to increasing rates of temperature fall in the early stages of a freeze is well illustrated by the buds on 12-inch and 18-inch twigs of Cumberland and Golden Jubilee. The more tender buds on 12-inch twigs show a significant difference one treatment higher in table 5 than the buds on the 18-inch twigs; for example, 12-inch twigs of Golden Jubilee react significantly between treatments 3 and 4, whereas the buds on the 18-inch twigs show a significant difference between treatments 4 and 5. Thus to obtain the most efficient results with this method it seems advisable to use the same starting temperature for the bath, especially if temperatures below 35° are used. In daily runs for hardiness determinations, samples have been started between 40° and 35° to insure a gradual cooling (not over 5° to 6° change per hour) in the first part of the freeze.

DATA TABLE 6

In experiment 6 also the rate of temperature fall is the same for all samples down to the low points at which they were removed from the bath. For each degree that the temperature fell below 0° F., an increase in injury to the fruit buds occurred. No significant difference between the percentage of buds killed at −7.0° and −7.5° F., or between −7.5° and −8.0° was apparent, but for all varieties except N. J. 66 a temperature of 1° lower within the critical range of temperatures caused significantly greater injury. Of the four varieties tested in Experiment 6 N. J. 66 has the tenderest fruit buds. Under the conditions of this artificial freeze carried out on February 25,

1941, there seemed to be one level at which large percentages of the buds of N. J. 66 were killed (cf. treatments 2 and 3, table 6), but both above and below this level there were no significant changes. When examined by the chi square test for homogeneity, a tendency toward a lack of uniformity in treatments 2 and 4 was indicated.

From table 6 it appears that if there is or is not a significant difference between two varieties treated similarly (removed at the same subzero temperature), these differences or likenesses will be evident until nearly complete bud killing occurs. As the lower temperatures are reached, buds of tender varieties are more severely injured than those of hardier varieties, a factor which tends to cause greater differences between varieties in certain treatments; for example, treatments 2, 3, and 4 in comparisons of Cumberland and Golden Jubilee.

DATA TABLE 7

The weather conditions of a single winter's night are known to have caused appreciable injury to peach fruits buds in New Jersey, as well as in other peach-growing districts. If it is assumed that little change in cold hardiness of the varieties studied occurred between February 25 and 27, 1941, then the data presented in tables 6 and 7 indicate that peach buds are somewhat more resistant to cold injury during a slow rate of freezing than during the usual rate of freezing. The apparent exception in Summercrest is explained by the fact that because of lack of available 12-inch and 18-inch twigs in the labeled trees sampled on previous dates, most samples collected on February 27, 1941, were taken from other trees of this variety. The fruit buds of these trees were relatively tender on that date, as judged by the results of experiment 7.

Samples of Cumberland were also difficult to obtain for experiment 7. Lack of uniformity in the Cumberland used in treatments 1 and 2 (table 7) was indicated by chi square tests for homogeneity. Little attention would be paid to the results of these treatments, except for the fact that the data agree well with the tendencies observed for other varieties, and through all the treatments. The chi square values in the comparisons between treatments 1 and 2 for Cumberland were large for 1 degree of freedom, the P values being well beyond the 1-percent point. With the slow freezing used in experiment 7 it was possible to subject live buds of the four varieties tested to much lower temperatures than they are known to have withstood under winter conditions in the orchard at New Brunswick. Although $-5°$ F. is a critical temperature (temperature following which varieties such as Elberta usually exhibit 30 percent or more fruit-bud injury) for dormant fruit buds of most varieties of peaches at New Brunswick, buds of Golden Jubilee collected at the college farm withstood $-11°$ F. during a slow rate of artificial freezing (an average of about $1.25°$ cooling per hour to $0°$) with relatively little more injury than was caused by $-6°$ during a moderate rate of artificial freezing (an average of about $2.5°$ to $2.8°$ cooling per hour to $0°$). These results agree with orchard observations over the country. In districts north of New Jersey where maximum winter temperatures are not so high as a rule, most varieties of peaches withstand somewhat lower winter temperatures than in New Brunswick.

SUMMARY AND CONCLUSIONS REGARDING DIRECT CONTROLLED FREEZING FOR HARDINESS DETERMINATIONS

Direct controlled artificial freezing of dormant fruit buds according to methods outlined herein provides a rapid and reliable method for estimating the relative cold hardiness of fruit buds of different varieties of peach. Relatively simple equipment that can be readily manipulated to simulate natural freezing conditions was used. Slow rates of cooling to subzero temperatures (average change of 2° to 3° F. per hour) were obtained by using a constantly stirred bath of antifreeze placed within the compartment of a commercial ice-cream refrigerator. As many as 50 samples could be frozen at a time. When varietal samples were so frozen artificially that only 1 to 15 percent of the buds of Elberta, a criterion variety, remained alive, other varieties tested had percentages of live buds that compared favorably with their previous response in orchards. Variations occurred in the hardiness of buds of the same variety on qualitatively different twigs. Because of this, care in the selection of samples must be practiced. Suggestions for sampling are given herein.

The data presented emphasize the importance of the rate of temperature fall in the development of cold injury to peach fruit buds. When the rate of cooling was the same, a drop of 1° F. within the critical subzero temperature range caused a significant increase in injury to peach fruit buds of the varieties tested. Under prolonged slow rates of cooling to subzero temperatures, peach buds were found to tolerate minimum temperatures appreciably below those that the same varieties are known to have withstood under orchard conditions at New Brunswick, N. J.

When using this controlled direct-freezing method for cold-hardiness determinations, it is advisable to include more than one criterion variety in each test. The response of these criterion varieties serve as standards for estimating the relative cold hardiness of the other varieties employed in the test. This procedure is a prerequisite to the use of the direct freezing method, since the temperatures prevailing in the orchard for several days before the collection of samples, and possibly other factors as well, may exert considerable influence on the cold hardiness of peach buds during the dormant season. The results obtained also indicate the advisability of testing the cold hardiness of a variety two or more times during the dormant season.

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PHYSIOLOGIC SPECIALIZATION IN THE TOMATO WILT FUNGUS FUSARIUM OXYSPORUM f. LYCOPERSICI [1]

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INTRODUCTION

In 1939 Bohn and Tucker [2] described the resistance of *Lycopersicon pimpinellifolium* Mill., accession 160, to the tomato wilt fungus, *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen. They [3] [4] studied the inheritance of resistance in *L. esculentum* Mill. $\times$ *L. pimpinellifolium* (acc. 160) hybrids and concluded that resistance was governed by a single, dominant Mendelian factor. The senior author of the present paper, working at the Ohio Agricultural Experiment Station, crossed *L. pimpinellifolium* (acc. 160) and *L. esculentum* var. Bonny Best, but inoculation experiments failed to duplicate the results reported by Bohn and Tucker. Two fusarium isolates were used. One came from a diseased plant in a glasshouse near Cleveland, Ohio, and the other was obtained from Dr. F. L. Wellman, of the United States Department of Agriculture.

In seeking an explanation for the divergent results, cultures of the fusarium were interchanged between the writers. Subsequent investigations carried on at both experiment stations show that the conspicuously different responses to inoculation were caused by marked differences in the pathogenicity of the isolates. Bohn and Tucker [5] and Wellman and Blaisdell [6] [7], as well as earlier investigators who tested the pathogenicity of isolates of the tomato wilt fungus, reported differences in the pathogenicity of the isolates studied. However, there are no reports known to the writers of the occurrence of physiologic specialization or physiologic races of *Fusarium oxysporum* f. *lycopersici* with the ability to infect and cause wilting of tomato varieties that are highly resistant to other isolates of the fungus known to be virulently pathogenic to susceptible varieties.

METHODS

The techniques used at the two experiment stations to test tomato plants for resistance to wilt were different, and, since the resulting incidence of disease differed somewhat when the same tomato lines and the same isolates of the wilt fungus were used, both methods are given in detail.

[2] BOHN, G. W., and TUCKER, C. M. IMMUNITY TO FUSARIUM WILT IN THE TOMATO. Science 89: 603–604. 1939.
[3] ——— and TUCKER, C. M. See footnote 2.
[4] ——— and TUCKER, C. M. STUDIES ON FUSARIUM WILT OF THE TOMATO. I. IMMUNITY IN LYCOPERSICON PIMPINELLIFOLIUM MILL. AND ITS INHERITANCE IN HYBRIDS. Mo. Agr. Expt. Sta. Res. Bul. 311, 82 pp., illus. 1940.
[5] BOHN, G. W., and TUCKER, C. M. See footnote 4.
[6] WELLMAN, F. L., and BLAISDELL, D. J. DIFFERENCES IN GROWTH CHARACTERS AND PATHOGENICITY OF FUSARIUM WILT ISOLATIONS TESTED ON THREE TOMATO VARIETIES. U. S. Dept. Agr. Tech. Bul. 705, 29 pp., illus. 1940.
[7] ——— and BLAISDELL, D. J. PATHOGENIC AND CULTURAL VARIATION AMONG SINGLE-SPORE ISOLATES FROM STRAINS OF THE TOMATO-WILT FUSARIUM. Phytopathology 31: 103–120, illus. 1941.

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The technique used at the Ohio station was as follows: Seedlings were grown in sterilized soil in flats for 4 to 6 weeks. Inoculum was prepared by growing the fungus in Petri dishes on potato-dextrose agar made with 200 gm. of potatoes and 1.75 percent each of dextrose and agar per liter of medium. When cultures were ready for use, the agar with the mycelial mat was scraped into a rotary fruit press and thoroughly macerated. Sufficient water was stirred into the agar-mycelium mixture to give the inoculum the consistency of a moderately thick soup. At the time of inoculation the plants were removed from the soil in the flats and the roots were washed. The roots were then dipped in the agar-water suspension of the fungus and transplanted. As the plants wilted and died, they were marked by short wire stakes. Records for resistance were taken when no more plants died, usually about 5 weeks after inoculation. When tests for wilt resistance were being conducted an effort was made to keep the air temperature of the glasshouse at 70° F.; however, the temperature fluctuated between 60° and 85°. The soil temperature of the test bed was much more constant and was maintained at about 80° by means of a soil-heating cable and thermostat.

The technique used at the Missouri station was as follows: The seeds were planted in sterilized soil in 8-inch clay pots. The inoculum was prepared by growing the fungus on sterile oats in pint jars and was about 25 days old when used. A water suspension of the oat cultures (1 pint of oats to 1 gallon of water) was poured over the seed at the rate of 300 ml. per pot before the seed was covered with soil. Twenty-five seeds were planted in each pot. At frequent intervals plants with definite symptoms of wilt were removed and the number recorded. Final records were taken 7 weeks after planting. In some experiments the seedlings were transplanted when 1 month old and inoculated by dipping the roots in a similar oat suspension; sometimes a second transplanting and inoculation were made 1 month later. The air temperature of the glasshouse was maintained at about 80° F. with little fluctuation.

The methods of recording disease resistance were similar at both locations. "Healthy" was used to describe plants with no external symptoms of the disease or vascular discoloration in the stem when cross-sectioned near the soil level. "Vascular discoloration" was used to describe plants with no external symptoms of the disease but with vascular discoloration. "Severely diseased" was used to describe plants which died from disease or which had severe symptoms of disease during the progress of the experiment.

Four isolates of the fusarium wilt fungus were used. These are designated as Ohio 39, Washington 5, and Missouri 6 and 10. Ohio 39 was isolated by the senior author in 1939 from a diseased tomato plant found at the Berea Greenhouse Co., Berea, Ohio. Washington 5 was furnished by Dr. F. L. Wellman and is probably the one described as No. 5 in the work of Wellman and Blaisdell.[8] If this supposition is correct, the isolate originally came from Bohn as Missouri isolate No. 7. Missouri Nos. 6 and 10 were reisolated from *Lycopersicon esculentum* × *L. pimpinellifolium* (acc. 160) hybrids inoculated with a mixture of cultures at Columbia, Mo. Single-spored cultures were not used. When mixed cultures of the fungus were used the isolates

8 WELLMAN, F. L., and BLAISDELL, D. J. See footnote 6.

were grown separately and mixed in equal proportions at the time of inoculation.

<div align="center">RESULTS</div>

<div align="center">TESTS WITH MIXED CULTURES</div>

Preliminary experiments for comparing the pathogenicity of a mixture of Missouri isolates 6 and 10 with that of a mixture of Washington 5 and Ohio 39 were performed at both experiment stations. The plants tested for resistance included the parental varieties Bonny Best and *Lycopersicon pimpinellifolium* (acc. 160), their F_1, F_2, and F_3 progenies; the backcross to Bonny Best and the F_2 of this backcross; F_2 progenies from crossing *L. pimpinellifolium* (acc. 160) with *L. esculentum* vars. Indiana Baltimore and Early Stone; and F_1 hybrids, Bonny Best $\times$ Accession 160, outcrossed to *L. esculentum* vars. Globe and Indiana Baltimore.

Bonny Best was completely susceptible in tests with both mixtures. *Lycopersicon pimpinellifolium* (acc. 160) was resistant to the Missouri mixture but segregated for resistance and susceptibility to the Ohio-Washington mixture. The latter mixture also caused a much higher incidence of disease in the F_1, F_2, and selfed backcross progenies. Six F_3 progenies and 6 selfed backcross progenies of the cross Bonny Best $\times$ *L. pimpinellifolium* (acc. 160) were tested for resistance. A total of 221 plants among the 6 F_3 progenies was tested for wilt resistance with the Missouri mixture. Ninety-one plants, or 41.2 percent, were diseased. A total of 214 plants was tested for resistance with the Ohio-Washington mixture. One hundred and thirteen, or 52.8 percent, were diseased. A chi-square test for homogeneity between the 2 groups gave the significant value $\chi^2 = 5.86$ ($df = 1$ $P < 5$ percent), thus indicating a difference in pathogenicity between the mixtures. Of the 6 selfed backcross progenies tested, a total of 234 plants was tested for resistance to the Missouri and 230 for resistance to the Ohio-Washington mixture. The former produced 128 diseased plants, or 54.7 percent infection, whereas the latter produced 166 diseased plants or 72.1 percent infection. A chi-square test for homogeneity between the percentage of diseased plants from the 2 groups gave the significant value $\chi^2 = 15.3$ ($df = 1$ $P < 1$ percent), again indicating a difference in pathogenicity between the 2 mixtures.

Selfed backcross and F_3 progenies which were homozygous for susceptibility to the Missouri mixture were likewise susceptible to the Ohio-Washington mixture. Progenies which segregated for resistance and susceptibility to the Missouri mixture also segregated for resistance to the Ohio-Washington mixture. However, the number of plants infected by the latter mixture was significantly greater than that infected by the former mixture. For example, among 10 segregating progenies inoculated with the 2 mixtures, the Missouri mixture produced disease in 70 plants among 369, or 19.0 percent infection, whereas the Ohio-Washington mixture produced disease in 157 plants among 347, or 45.2 percent infection. A chi-square test for homogeneity between the two groups gave the significant value $\chi^2 = 57.0$ ($df = 1$ $P < 1$ percent), indicating a difference in pathogenicity between the 2 mixtures. Progenies homozygous for resistance to the Missouri mixture segregated for resistance and susceptibility to the Ohio-Washington mixture.

It appeared that at least one of the isolates in the Ohio-Washington mixture possessed a type of pathogenicity which differed from that of the Missouri isolates and that the factor which governed resistance to Missouri isolates was at most only partly effective against one or both of the isolates in the Ohio-Washington mixture.

The results of the preliminary experiments at Ohio and Missouri were in general agreement, although the number of diseased plants was usually greater in Ohio, where the Ohio method of inoculation was used.

TESTS WITH INDIVIDUAL CULTURES

Certain of the parent varieties and F_1 and F_2 hybrid generations (Table 1) were tested for susceptibility to the four isolates separately in Ohio and Missouri. The varieties Bonny Best and Globe were very susceptible in each test. *Lycopersicon pimpinellifolium* (acc. 160) and the F_1 hybrid progeny with Bonny Best proved highly resistant to Missouri isolates 6 and 10 at both locations. With isolate Washington 5 the incidence of disease was higher than with the Missouri isolates in the resistant parent and in the F_1 generation progeny. With the Ohio isolate, the incidence of disease was significantly greater than with the other three isolates.

TABLE 1.—*Pathogenicity of individual cultures of Fusarium oxysporum f. lycopersici*

Pedigree [1]	Location	Missouri No. 6				Missouri No. 10				Washington No. 5				Ohio No. 39			
		Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased
		No.	No.	No.	Pct.	No.	No.	No.	Pct.	No.	No.	No.	Pct.	No.	No.	No.	Pct.
Parental varieties																	
Bonny Best	Missouri	0	0	29	100.0	0	0	26	100.0	0	0	32	100.0	0	0	35	100.0
Bonny Best	Ohio	0	1	71	100.0									0	1	23	100.0
Globe	do	2	1	45	95.8	0	1	23	100.0	1	1	46	97.9	1	0	39	97.5
Acc. 160	Missouri	35	1	0	2.8	38	0	0	0	38	0	2	5.0	20	20	8	58.3
Acc. 160	Ohio	24	0	0	0	24	0	0	0	18	1	5	25.0	4	3	10	76.5
F_1 *Generation*																	
(BB × Acc. 160)	Missouri	14	0	0	0	13	0	0	0	9	2	0	18.2	3	4	2	66.7
(BB × Acc. 160)	Ohio	9	3	1	30.8	8	5	0	38.5	4	4	5	69.2	2	2	9	84.6
F_2 *Generation*																	
(BB × Acc. 160)	Missouri					36	6	4	21.7	36	3	6	20.0	36	4	8	25.0
(IB × Acc. 160)	do					265	22	36	18.0	244	18	59	24.0	202	50	94	41.6
(IB × Acc. 160)	Ohio	14	4	6	41.7	11	3	10	54.2	11	3	8	50.0	1	2	20	95.7
(ES × Acc. 160)	Missouri					247	27	42	21.8	208	21	57	27.3	185	48	84	41.6

[1] Abbreviations: BB = Bonny Best; IB = Indiana Baltimore; ES = Early Stone; Acc. 160 = *Lycopersicon pimpinellifolium* (acc. 160).

The reactions of the F_2 progenies were similar, as shown in table 1, with respect to the Missouri and the Washington isolates, but the Ohio isolate again caused a decidedly higher percentage of diseased plants. When the number of healthy plants and the number of diseased plants in the four F_2 progenies resulting from inoculation with the three cultures, Missouri 10, Washington 5, and Ohio 39, were compared separately for homogeneity the chi-square values 0.35, 50.34, 13.72, and 31.23 were obtained. At the 1-percent point with two degrees of freedom, only one progeny (BB $\times$ Accession 160) appeared to be homogeneous in its reaction to the three cultures. The other three progenies were significantly heterogeneous in their reactions. These three progenies appeared to react similarly to the cultures Missouri 10 and Washington 5, but differently to Ohio 39. Only one of the four F_2 progenies, when inoculated with the Ohio 39 culture, appeared to segregate for resistance and susceptibility according to a 3:1 ratio. When the number of healthy and diseased plants in the other progenies were compared individually for goodness of fit to a 3:1 ratio, the chi-square values 50.96, 64.22, and 46.81 were obtained, indicating significant deviations. It is not known why the F_2 progeny (BB $\times$ Accession 160) reacted similarly to all three cultures, especially since the F_1 and F_3 progenies of the same cross reacted so differently (tables 1 and 2).

TABLE 2.—*Pathogenicity of individual cultures of Fusarium oxysporum f. lycopersici to selfed backcross progenies, tested at Wooster, Ohio*

Pedigree[1]	Cultures tested											
	Missouri No. 6				Washington No. 5				Ohio No. 39			
	Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased
	No.	*No.*	*No.*	*Pct.*	*No.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*No.*	*Pct.*
[(BB×Acc. 160)×BB]—17	0	2	22	100.0	0	0	23	100.0	1	1	22	95.8
[(BB×Acc. 160)×BB]—1	1	0	23	95.8	0	1	24	100.0	0	0	16	100.0
[(BB×Acc. 160)×BB]—18	1	2	21	95.8	0	3	21	100.0	0	5	19	100.0
[(BB×Acc. 160)×BB]—20	1	2	18	95.2	0	0	20	100.0	0	2	19	100.0
[(BB×Acc. 160)×BB]—10	2	0	22	91.7	1	0	23	95.8	2	0	14	87.5
[(BB×Acc. 160)×BB]—3	2	0	22	91.7	2	1	21	91.7	1	0	15	93.8
[(BB×Acc.·160)×BB]—11	3	0	21	87.5	2	2	20	91.7	3	0	13	81.3
[(BB×Acc. 160)×BB]—4	3	1	20	87.5	0	1	23	100.0	2	0	14	87.5
[(BB×Acc. 160)×BB]—8	5	1	18	79.2	1	1	22	95.8	1	0	15	93.8
[(BB×Acc. 160)×BB]—6	9	5	10	62.5	14	5	5	41.7	4	2	10	75.0
[(BB×Acc. 160)×BB]—2	13	4	7	45.8	12	2	10	50.0	6	2	8	62.5
[(BB×Acc. 160)×BB]—5	13	3	8	45.8	12	6	6	50.0	0	1	15	100.0
[(BB×Acc. 160)×BB]—13	13	1	10	45.8	13	1	10	45.8	1	1	14	93.8
[(BB×Acc. 160)×BB]—15	13	4	7	45.8	10	4	10	58.3	3	2	19	87.5
[(BB×Acc. 160)×BB]—21	13	5	6	45.8	13	3	7	43.5	1	2	21	95.8
[(BB×Acc. 160)×BB]—14	16	2	6	33.3	19	0	5	20.8	4	0	12	75.0
[(BB×Acc. 160)×BB]—9	17	4	3	29.2	10	1	13	58.3	3	0	13	81.3
[(BB×Acc. 160)×BB]—12	17	2	5	29.2	19	1	4	20.8	4	1	11	75.0
[(BB×Acc. 160)×BB]—16	17	2	5	29.2	16	2	6	33.3	4	3	20	95.8
[(BB×Acc. 160)×BB]—7	19	3	2	20.8	15	4	5	37.5	2	0	14	87.5
Total or average	178	43	256	62.7	159	38	278	66.5	39	22	304	89.3

[1] Abbreviations: BB=Bonny Best; Acc. 160=*Lycopersicon pimpinellifolium* (acc. 160).

The results obtained when the three isolates, Missouri 6, Washington 5, and Ohio 39 were tested for pathogenicity against 20 selfed backcross progenies are shown in table 2. The mean percentages of

diseased plants resulting from inoculation with the Missouri and Washington cultures were similar, 62.7 and 66.5, respectively. The mean percentage of diseased plants caused by the Ohio isolate, 89.3, was decidedly higher. The increased percentage of diseased plants caused by the Ohio culture occurred in the segregating progenies and is particularly noticeable in progenies 5, 7, 9, 12, 13, 14, 15, 16, and 21. These segregated for resistance and susceptibility to the Missouri

FIGURE 1.—Results of inoculations with Missouri isolate No. 6 (*A*) and Ohio isolate No. 39 (*B*) on Bonny Best, BB, and two selfed backcross progenies No. 1, susceptible, and No. 7, segregating for resistance to the Missouri isolate. Note that Bonny Best was completely susceptible in tests with both isolates.

and Washington isolates but were generally susceptible to the Ohio isolate. Among the progenies segregating for resistance and susceptibility to the Missouri and Washington isolates, the Ohio isolate caused 85.5 percent diseased plants.

Progenies 1, 3, 4, 8, 10, 11, 17, 18, and 20 were apparently homozygous for susceptibility to the Missouri and Washington isolates and were likewise highly susceptible to the Ohio isolate. The percentages of diseased plants caused by Missouri 6, Washington 5, and Ohio 39 were 91.5, 97.3, and 93.9, respectively.

The number of healthy and diseased plants in the 20 selfed backcross progenies inoculated with Ohio isolate 39 did not vary greatly. However, when a chi-square test for homogeneity was made, the significant value $\chi^2 = 38.27$ ($df = 19$ $P < 1$ percent) was obtained, indicating that the progenies did not segregate uniformly. The results show that segregation occurred within some of the individual progenies but are inconclusive as to whether the ratio of diseased and healthy plants differed between the progenies. A photograph showing the reaction of two progenies (No. 1, susceptible, and No. 7, segregating) in tests for resistance to two isolates, Missouri No. 6 and Ohio No. 39, is presented in figure 1.

TABLE 3.—*Pathogenicity of individual isolates of Fusarium oxysporum f. lycopersici to advanced generation progenies, tested at Columbia, Mo.*

Progeny [1]	Cultures tested															
	Missouri No. 6				Missouri No. 10				Washington No. 5				Ohio No. 39			
	Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased
	No.	No.	No.	Pct.	No.	No.	No.	Pct.	No.	No.	No.	Pct.	No.	No.	No.	Pct.
PROGENIES HOMOZYGOUS FOR RESISTANCE TO MISSOURI AND WASHINGTON CULTURES																
10-1085-9	36	0	0	0	46	0	0	0	38	0	0	0	24	12	7	44.2
10-1005-3	40	0	0	0	46	0	0	0	38	1	0	2.6	11	18	12	73.2
10-703-14	45	0	0	0	38	0	0	0	36	0	0	0	10	14	15	74.4
Total or average	121	0	0	0	130	0	0	0	112	1	0	0.9	45	44	34	63.4
PROGENIES HOMOZYGOUS FOR SUSCEPTIBILITY TO MISSOURI AND WASHINGTON CULTURES																
10-105-7	0	0	42	100.0	0	0	42	100.0	0	1	39	100.0	1	0	43	97.7
10-644-6	1	0	15	93.8	0	0	16	100.0	0	0	19	100.0	1	1	17	94.7
M-140-5-1	0	0	38	100.0	1	0	38	97.4	0	0	44	100.0	1	1	45	97.9
Total or average	1	0	95	99.0	1	0	96	99.0	0	1	102	100.0	3	2	105	97.3

[1] Pedigrees: 10-1085-9. E × Acc.160 × BD × Bis × Bis × Bis, s, × GB, fourth self.
10-1005-3. E × Acc.160 × BD × Bis × Bis × BD, fifth self.
10-703-14. E × Acc.160 × BD, s, × Pond, s, s, × GB, fourth self.
10-105-7. BB × Acc.160 × BB × BB × WB, fourth self.
10-644-6. E × Acc.160 × BD × Bis × Bis, s, s, × EB, fourth self.
M-140-5-1. E × Acc.160 × BD × BB × Pond, fourth self.
Abbreviations: BB=Bonny Best; BD=Break O'Day; Bis=Bison; E=Earliana; EB=Early Baltimore; GB=Greater Baltimore; Acc.160=*Lycopersicon pimpinellifolium* (acc. 160); Pond=Ponderosa; WB=White Beauty; s=selfed.

Table 3 embodies results obtained from inoculation of six advance generation progenies, three homozygous for resistance and three homozygous for susceptibility to the Missouri isolates. The progenies homozygous for susceptibility to the Missouri isolates proved equally

susceptible to the other isolates. The three resistant progenies were highly resistant to the Missouri and Washington isolates but segregated for resistance and susceptibility to the Ohio isolate. This segregation does not appear to be uniform and a chi-square test for homogeneity gave the significant value $\chi^2 = 10.57$ $(df = 2 \ P < 1$ percent), which suggests that the progenies are different genotypes. The difference appears to be due to the progeny 10–1085–9. The other two progenies appear to be homogeneous.

Ten advanced-generation progenies, 5 homozygous and 5 heterozygous for resistance to the Missouri isolates, selected on the basis of their behavior in glasshouse and field tests during the previous season, were compared for resistance to isolates Missouri 10 and Ohio 39. The plants inoculated with the Ohio isolate were grown to maturity. The results, presented in table 4, show that of the 5 progenies homozygous for resistance to the Missouri isolate 3 segregated for resistance and susceptibility to the Ohio isolate and 2 were completely susceptible. Four of the progenies heterozygous for resistance to the Missouri cultures were also heterozygous for resistance to the Ohio culture, and 1 was completely susceptible.

The pathogenicity of Ohio isolate 39 appears to differ qualitatively from that of the other isolates. When susceptible commercial varieties or hybrid progenies homozygous for susceptibility to the Missouri isolates were inoculated with Ohio 39 the resultant disease symptoms did not develop earlier or with greater severity than when these progenies were inoculated with the Missouri isolates. Indeed, the Ohio isolate would have been regarded as slightly less virulent than the Missouri and Washington isolates had the inoculations been confined to such genotypes. It was only when progenies with the factor for resistance to the Missouri and Washington isolates obtained from *Lycopersicon pimpinellifolium* (acc. 160) were inoculated that the unusual pathogenicity of the Ohio isolate became evident.

Other commercial varieties tested proved susceptible to Ohio 39. For example, in a test terminated 7 weeks from planting, Break O'Day, Rutgers, and Pan America became diseased to the extent of 86, 81, and 71 percent, respectively. The variety Pan America, described by Porte and Walker [9] in 1941, was developed from the cross *Lycopersicon pimpinellifolium*, P. I. 79532, (possibly identical with Bohn and Tucker's Accession 160) × Marglobe, with backcrosses to Marglobe. The variety proved highly resistant to the Missouri isolates but only slightly resistant to Ohio 39.

The four isolates, Missouri 6 and 10, Washington 5, and Ohio 39, were compared in culture on potato-dextrose, maize-meal, and oatmeal agars. They proved similar in growth rates, in temperature-growth relations, and in the morphology of micro- and macrocinidia. The isolates varied in certain growth characters. Some produced abundant aerial mycelium and caused little coloration in the medium. With others the mycelium was appressed to the surface of the agar, which assumed various shades of lavender or purple. Ohio 39 produced saltant sectors in potato-dextrose-agar cultures. Transfers from the sectors yielded lines with various growth types. The behavior of the

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[9] PORTE, W. S., and WALKER, H. B. THE PAN AMERICA TOMATO, A NEW RED VARIETY HIGHLY RESISTANT TO FUSARIUM WILT. U. S. Dept. Agr. Cir. 611, 6 pp., illus. 1941.

four isolates in culture was in accord with the results reported by Wellman and Blaisdell [10] in a study of a collection of isolates of the wilt fungus.   From these observations the four isolates appear to be *Fusarium oxysporum* f. *lycopersici*.

TABLE 4.—*Pathogenicity of Missouri isolate No. 10 and Ohio isolate No. 39 to advanced generation progenies in tests at Columbia, Mo.*

[Seeds planted Mar. 7, 1942; plants transplanted and inoculated Mar. 28 and again May 15. Plants inoculated with No. 10 removed May 15; plants inoculated with Ohio 39 grown to Nov. 11, 1942]

Progeny [1]	Cultures tested														
	Missouri No. 10					Ohio No. 39									
	Wilt to May 15					First inoculation Mar. 28, 1942			Plants lost transplanting	Second inoculation May 15, 1942, records taken Nov. 11, 1942					Total diseased both transplantings
	Plants	Healthy	Vascular discoloration	Seriously diseased	Total diseased	Plants	Diseased	Diseased		Transplanted	Healthy	Vascular discoloration	Seriously diseased	Total diseased	
	No.	No.	No.	No.	Pct.	No.	No.	Pct.	No.	No.	No.	No.	No.	Pct.	Pct.
LINES HOMOZYGOUS FOR RESISTANCE TO MISSOURI ISOLATES															
10–25–1	79	79	0	0	0	78	46	59.0	2	30	7	7	16	76.7	90.8
10–26–3	79	77	1	1	2.5	80	69	86.3	3	8	0	0	8	100.0	100.0
10–31–4	80	80	0	0	0	80	69	86.3	2	9	0	1	8	100.0	100.0
10–32–1	75	75	0	0	0	79	43	54.4	3	33	12	10	11	63.6	84.2
10–38–1	80	80	0	0	0	79	24	30.4	2	53	27	11	15	49.1	64.9
Total or average	393	391	1	1	.5	396	251	63.4	12	133	46	29	58	65.4	88.0
LINES HETEROZYGOUS FOR RESISTANCE TO MISSOURI ISOLATES															
10–6–8	85	76	4	5	10.6	78	63	80.8	3	12	3	4	5	75.0	96.0
10–33–12	80	66	6	8	17.5	80	48	60.0	0	32	18	11	3	43.7	77.5
10–41–5	76	71	1	4	6.6	81	63	77.8	5	13	6	2	5	53.8	92.1
10–43–4	78	74	1	3	5.1	80	62	77.5	5	13	3	0	10	76.9	96.0
10–70–4	78	75	2	1	3.8	81	73	90.1	6	2	0	1	1	100.0	100.0
Total or average	397	362	14	21	8.8	400	309	77.2	19	72	30	18	24	58.3	92.1

[1] Pedigrees: 10–25–1, 10–26–3, 10–31–4, 10–32–1, 10–38–1. BB × Acc. 160 × BB × BB × BD, third self.
10–6–8. BB × Acc. 160 × Ox × Bis × BD, third self.
10–33–12. BB × Acc. 160 × BB × BB × BD, third self.
10–41–5, 10–43–4. BB × Acc. 160 × BB × BB × BD, s, × ES, first self.
10–70–4. BB × BB × (BB × Acc. 160), s, × ES, first self.
Abbreviations: BB=Bonny Best; BD=Break O'Day; Bis=Bison; ES=Early Stone; Acc. 160=*Lycopersicon pimpinellifolium* (acc. 160); Ox=Oxheart; s=selfed.

DISCUSSION

The Missouri and Washington isolates exhibited the same type of pathogenicity, although the latter consistently caused a few more diseased plants.   On the other hand, the Ohio isolate appeared to possess an entirely different type of pathogenicity.   It proved pathogenic to *Lycopersicon pimpinellifolium* (acc. 160) and caused signifi-

[10] WELLMAN, F. L., and BLAISDELL, D. J. See footnote 6.

cantly higher percentages of diseased plants in the F_1, F_2, and back-cross progenies, and in advanced generations selected for resistance to the Missouri isolates. Resistance to the Missouri and Washington isolates appeared to segregate according to a simple Mendelian ratio, when tested at Missouri with the Missouri technique; the inheritance of resistance to the Ohio isolate appeared to be more complicated, regardless of where the tests were made, as shown by the high percent-age of diseased plants. The Ohio isolate caused much larger per-centages of infected plants in some of the selfed backcross progenies than did the Missouri or Washington isolates (table 2). The differ-ences in susceptibility indicated that tomato lines highly resistant to the Missouri and Washington isolates might be very susceptible to the Ohio isolate. Some of the progenies apparently homozygous for resistance to the Missouri isolates, proved highly susceptible to the Ohio isolate (table 4).

Since all progenies homozygous for susceptibility to the Missouri and Washington isolates were likewise susceptible to the Ohio isolate, it appears that the absence of the factor for resistance to the former results in susceptibility to the Ohio isolate. Some progenies homozy-gous for resistance to the Missouri and Washington isolates were completely susceptible to the Ohio isolate, whereas others segregated for resistance and susceptibility. The results suggest that the factor for resistance to the Missouri and Washington isolates must be present if there is to be resistance to the Ohio isolate, but that this factor alone is not sufficient to assure resistance to it. The presence of an undeter-mined number of complementary resistance factors, in addition to the factor for resistance to the Missouri and Washington isolates, is probably necessary for resistance to the Ohio isolate. If this is true it is to be expected that tomato lines homozygous for resistance to the Missouri and Washington isolates should exhibit various degrees of resistance, or complete susceptibility to the Ohio isolate, depending on the presence and the number of complementary factors in the genotype.

The advanced generations tested for resistance (tables 3 and 4) were developed at the Missouri station and were obtained by backcrossing to the susceptible parent, outcrossing to commercial varieties, several generations of selfing, and with selection always directed toward resistance to the Missouri isolates. If complementary resistance factors are necessary and enter the genotype from the *Lycopersicon pimpinellifolium* parent, it is not surprising that some or all of them were lost, resulting in lines homozygous for resistance to the Missouri isolates but heterozygous for resistance or homozygous for suscepti-bility to the Ohio isolate.

The demonstration of physiologic races of the fungus complicates the problem of development of varieties of tomatoes homozygous for resistance to all forms of the fusarium wilt organism. However, it does not appear to preclude the possibility of developing varieties that are highly resistant to all forms. This is brought out particularly in table 4, where it is shown that some resistance to Ohio 39 is retained even after several backcrosses to susceptible varieties without selec-tion for resistance to it.

Little is known regarding the prevalence or distribution of physi-ologic races of *Fusarium oxysporum* f. *lycopersici*. Such evidence as

is available indicates that they are not common. Bohn and Tucker,[11] in tests with 39 isolates, and Wellman and Blaisdell,[12] in tests with 29 isolates, found *Lycopersicon pimpinellifolium* to be very resistant to all. So far as the writers are aware, Pan America has proved consistently resistant in various areas. Selections from hybrids developed at Missouri, resistant to the Missouri isolates, proved resistant when grown in infested fields in Indiana, Alabama, Louisiana, Texas, California, Kansas, and South Africa.

The method of inoculation used at Ohio gave a higher percent of diseased plants than the method used at Missouri. However, this difference can probably be accounted for by the shock and root injury produced by the former method. Regardless of the fact that the incidence of disease was higher in the Ohio tests, the results secured at both locations appear to show that the Ohio isolate has a different type of pathogenicity from that of the Missouri and Washington isolates.

SUMMARY

A race of *Fusarium oxysporum* f. *lycopersici* has been isolated in Ohio which appears to have a distinctive type of pathogenicity.

A comparison of the pathogenicity of isolates from various sources revealed that tomato lines resistant to isolates of the types previously studied may be completely susceptible to the Ohio isolate.

The inheritance of resistance to the Ohio race appears to involve the gene for resistance to the Missouri race and an undetermined number of complementary genes.

Lycopersicon pimpinellifolium, accession 160, appears to be segregating for resistance and susceptibility to the Ohio race, possibly due to heterozygosity for the complementary resistance factor or factors.

All commercial varieties of tomato tested were susceptible to the Ohio isolate.

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[11] BOHN, G. W., and TUCKER, C. M. See footnote 4.
[12] WELLMAN, F. L., and BLAISDELL, D. J. See footnote 6.

TIP BLIGHT OF SPECIES OF ABIES CAUSED BY A NEW SPECIES OF REHMIELLOPSIS [1]

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INTRODUCTION

In 1933 a disease of the current season's growth of white, or Colorado, fir (*Abies concolor* (Gord.) Engelm.) was reported (*27*)[3] as the cause of extensive injury in a planting of about 500 trees in eastern Massachusetts and on a few ornamental trees of the same species at Augusta and Portland, Maine, and at Lake George, N. Y. The disease was attributed to *Rehmiellopsis bohemica* Bub. and Kab., which previously had been reported from Denmark (*21, 22*), Norway (*13*), Bohemia (*2*), and Scotland (*28*) on various European species of *Abies* and on the American species *A. nobilis* Lindl. Certain morphological differences, particularly in the size of the asci and spores, were noted between the fungus found in the eastern part of the United States and the species reported by European investigators. These differences were considered of sufficient importance to warrant a detailed study, with the result that the fungus occurring in this country is now described as a new species of *Rehmiellopsis.*

Because the disease was first observed in the Eastern States in ornamental plantings of white fir, it was thought that it might have been introduced into this country on horticultural material of other species of *Abies* from Europe. However, the determination of the causal fungus as a new species of *Rehmiellopsis* and the occurrence of the disease on balsam fir (*Abies balsamea* (L.) Mill.) in Maine (*26*) suggest that the fungus is native in the eastern part of the United States and may have spread from the native balsam firs to the introduced white firs used as ornamentals in New England and New York. The disease has not been found on white fir in the Western States, its native range. Preliminary surveys of areas of infection and experiments with cross infection of various species of *Abies* under natural conditions have already been reported (*26*). Additional and more conclusive data on these points are reported in this paper.

The disease varies in severity from one season to another and seems to be of relatively slight economic importance on the native balsam fir. Infection on the white firs destroys their value as ornamentals, for which they are used extensively in the eastern part of the United

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States, and has brought forth a demand by tree owners for information on control measures.

The present study includes the symptoms of the disease, the hosts and distribution, the morphology, taxonomy, and pathogenicity of the causal fungus, and the control of the disease on ornamentals.

SYMPTOMS

The earliest symptom of the disease appears on needles of the current season's growth when the bud scales begin to slough off. Several of the needles from a bud may show yellowish-pink spots on the tissue recently uncovered by the loosening bud scales. The young developing twig bearing these needles usually continues to grow, but before reaching mature size it turns dark brown or black and becomes shriveled, slightly curved, and brittle. All the needles on such a twig are affected by the fungus and change in color from light green to yellowish pink, then to dark reddish brown, and finally to gray. As the color changes and the leaf tissue dries out, the leaf margins roll backward toward the lower surface so that diseased needles appear distinctly narrower than healthy ones. They are also curved and bent out of their normal pattern of growth (fig. 1, *C*). The injury may easily be mistaken for frost injury (fig. 1, *B*), but in the latter case the reddish-brown needles appear slightly water-soaked and the growth of the young twig is immediately arrested.

It frequently happens that the twigs and needles have almost completed their development before infection takes place. In such cases most of the needles that develop later become discolored but only the tips of the twigs atrophy and die. Adventitious buds (fig. 1, *A*) may develop below the atrophied tip, producing weak, stunted needles late in the season. In many cases, however, the twigs remain uninfected and have normal terminal buds, although occasional needles or tips of needles may show characteristic color changes indicating infection. These needles usually have just attained mature size before infection, and the tissue, being soft, dries out rapidly after invasion by the fungus. The infected needles are very brittle, but most of them overwinter on the twigs for at least one season and sometimes two. On 1- and 2-year-old twigs, blackened leaf scars indicate where infected needles have broken off. On *Abies concolor* small cankers may form around leaf scars where the fungus has entered the twig tissue from an infected needle. These cankers have not been observed on *A. balsamea*.

About a month or 6 weeks after the first evidence of infection, small, black fruiting bodies are found in the tissue of the upper leaf surface, particularly along the curled leaf margins. These fruiting bodies develop very slowly during the summer, becoming slightly erumpent, but they do not reach maturity until the following spring when the new growth on the fir trees is developing. They form also on the brown, shriveled twigs and on the small cankers at the base of infected needles.

The disease appears first on the lower branches, and usually the lateral twigs of such branches are more severely affected than the terminal twigs. These laterals may be killed back to the node, while the terminal twigs may have only scattered infected needles. On seedling trees of balsam fir about 2 or 3 feet high in areas of infection in the native stands, all the needles of the new growth over

the entire tree and many of the twigs may be affected in one season. The following year the terminal buds on uninfected twigs will put out a weak, stunted growth, which, under weather conditions favor-
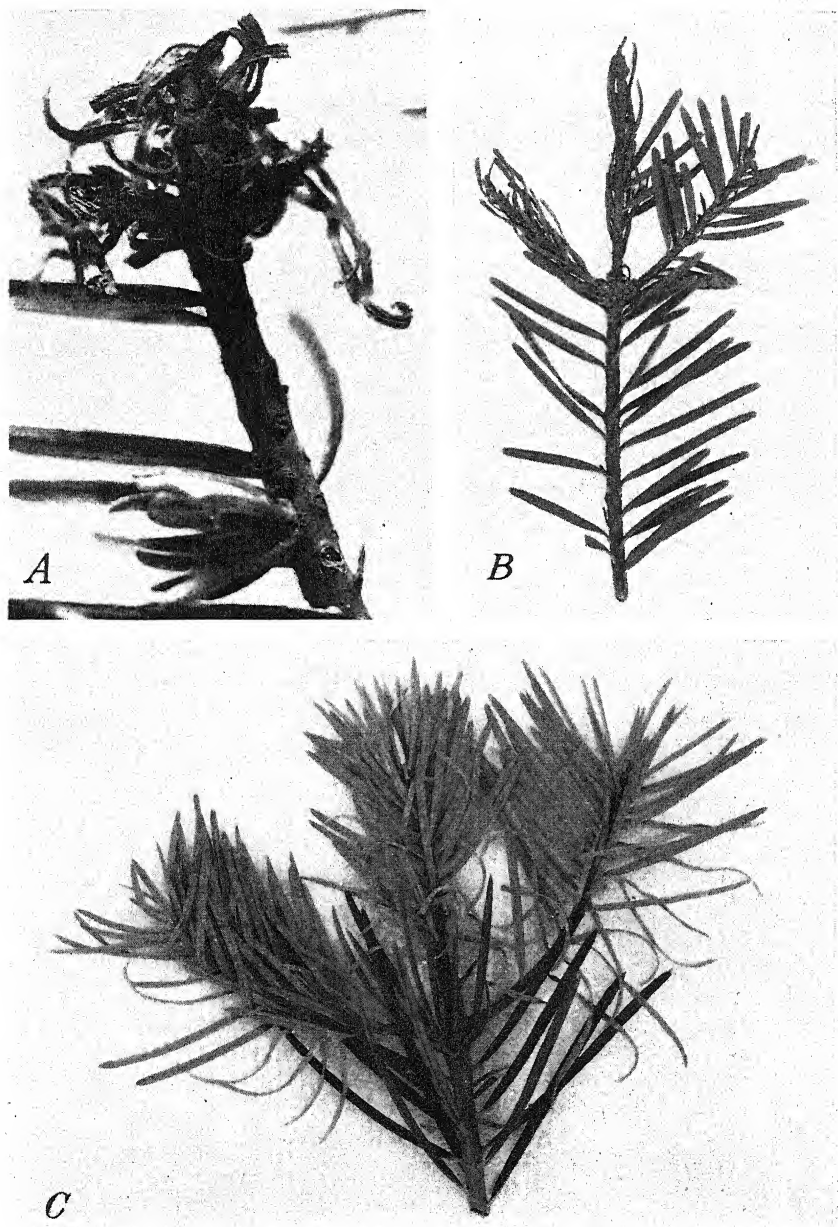
FIGURE 1.—Needles and twigs of species of *Abies* infected with *Rehmiellopsis balsameae: A, A. concolor,* twig with adventitious bud developed below infected area (approximately × 2); *B, A. balsamea,* showing dieback of young twigs (approximately × 1); *C, A. concolor,* with curled, drooping needles indicative of infection by *Rehmiellopsis balsameae* (approximately × 1).

able for the fungus, may become infected. Severe infection repeated for several years in succession results in the death of such trees. The spread of the fungus is so largely dependent upon seasonal growth and moisture, however, that if two or three seasons of heavy infection are followed by several seasons of light infection, the trees may recover to a remarkable degree. This is particularly true of older trees, which sometimes seem to be almost killed by the disease but exhibit a striking resumption of growth during seasons of light infection. For this reason, in the native stands of balsam fir the disease has not yet become serious and on ornamental trees control measures can be effectively employed.

Weather conditions seem to exert an important influence on the time of infection. In central and southern New England the spores reach maturity during the latter part of May and early June, when the buds of the white firs are normally developing. On balsam firs in northern Maine the season of bud development and infection is slightly later, usually occurring in late June or early July. An early growing season or one with limited rainfall results in a relatively small amount of bud and twig infection, so that only scattered needles of the new growth are injured. A delayed, moist season, on the other hand, causes a rapid development of young succulent tissue that is particularly susceptible to infection at a time when the fungus spores are mature. In such cases conspicuous and serious injury may occur within 2 weeks from the time that the bud scales are entirely sloughed off.

Fir trees of all sizes and ages may be affected. White firs about 40 years old have shown infection for the past 10 years with varying degrees of severity from one year to another, depending upon weather conditions, but with no apparent decrease in susceptibility as the trees increase in age. On native balsam firs over 30 years old, needles on all branches and on the leaders may become infected. In contrast to this, Wilson and MacDonald (*28*), in their study of a similar disease caused by *Rehmiellopsis bohemica*, found that in Scotland large trees of *Abies alba* Mill. 50 to 80 years old were not attacked by the disease even in the vicinity of heavily infected young plantations, and E. Rostrup (*21*) stated that in Denmark trees of *A. alba* 10 to 20 years old were susceptible to the same disease but trees about 30 years old seemed to be resistant.

HOSTS AND DISTRIBUTION

In a previous report of the distribution of the disease (*26*) the writer designated the causal fungus in all cases as *Rehmiellopsis bohemica*. The results of the present study, however, indicate that in the collections described in that report only the fungus on native *Abies lasiocarpa* (Hook.) Nutt., collected at Edgewood, British Columbia, is identical with the European species of *Rehmiellopsis*. The new species described in this paper occurs in the collections reported from native *A. balsamea* in northern Maine; from ornamental trees of *A. concolor* in southern Maine and New Hampshire, eastern Massachusetts, Rhode Island, and eastern New York; of *A. fraseri* (Pursh) Poir. and *A. nobilis* in eastern Massachusetts; and of *A. cephalonica* Loud. in Rhode Island.

Surveys in northern New England in the spring of 1940 and 1941 disclosed the presence of infected balsam firs in localities in northern

Maine additional to those already reported.[4] The area of infection extends from Ripogenus Dam in northeastern Maine to the New Hampshire border. Considerable infection was found also in the latter State from Colebrook to the Canadian border and just over the New Hampshire line in Vermont. Efforts have repeatedly been made to trace the disease southward toward the coast of Maine and New Hampshire, where areas of infection on white fir are located. Only one instance is known of the occurrence of infection on the two species of fir within a short distance of each other. In 1940 an infected balsam fir was found by C. K. Goodling about 10 miles east of Augusta, Maine, where white firs have been infected for at least 10 years. An inspection of other balsam firs in the region surrounding Augusta has failed to show any further evidence of infection. Moreover, a relatively thorough inspection of native balsam firs in New York State just north of infected white firs at Lake George revealed no evidence of the disease in 1937. It is impossible at present to account for these isolated spots of infection on white firs, but unknown sources may be located in the balsam firs on heavily wooded hills and mountains of southeastern Maine and northeastern New York.

In 1920 Faull (7) described a twig blight of balsam fir in Canada, the cause of which was then unknown. His description of the symptoms and his illustrations, however, give every indication that the disease was similar to or identical with that on balsam fir in Maine. Collections made by him in 1919 on Bear Island, Lake Timagami, Ontario, and in 1928 in the region of Ste. Anne des Monts, and Claude Lake, Gaspé County, Quebec, examined by the writer, showed characteristic symptoms and a few immature fruiting bodies but no spores. Two other collections, made by G. D. Darker in 1925 and J. R. Hansbrough in 1935 on Bear Island, Lake Timagami, also had immature fruiting bodies. All attempts to locate material from Quebec and Ontario with mature fruiting bodies have failed. It seems probable, however, that the fungus in these regions is identical with that across the border in Maine.[5]

MORPHOLOGY OF THE CAUSAL FUNGUS

HYPHAE

In a newly infected needle, the hyphae are found first in the intercellular spaces below the stomata, but the exact method of entrance by the germ tubes has never been observed. The hyphae advance between the mesophyll cells; as the leaf tissue gradually becomes affected by the fungus, the hyphae entirely fill the cells, even those of the vascular tissue. The newly developing hyphae are hyaline to subhyaline, measuring 4μ to 6μ in width, increasing to 14μ, and are densely granular. As the hyphae increase in width the walls become heavier. In late autumn, when the fruiting bodies have formed, the older hyphae throughout the tissue gradually turn brown and develop distinctly heavy walls and conspicuous oil globules.

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[4] WATERMAN, A. M., and ALDRICH, K. F. REHMIELLOPSIS NEEDLE BLIGHT OF BALSAM FIR IN MAINE. U. S. Bur. Plant Indus., Plant Dis. Reptr. 24:201-205, illus. 1940. [Processed.]

[5] A specimen of *Abies balsamea*, collected in July 1944 by W. A. Reeks at Cape Breton Island, Nova Scotia, and referred to the writer by Mildred K. Nobles, Central Experimental Farm, Ottawa, Canada, had a few infected needles of the previous year's growth with mature spores typical of the species of *Rehmiellopsis* that occurs in Maine.

PERFECT STAGE

DEVELOPMENT OF ASCOMA

The fruiting bodies of the ascogenous stage begin to form about 4 weeks after the needles become infected. At first they are composed of more or less globoid, loosely woven masses of hyaline hyphae and lie just below the epidermis, engulfing mesophyll cells in the vicinity
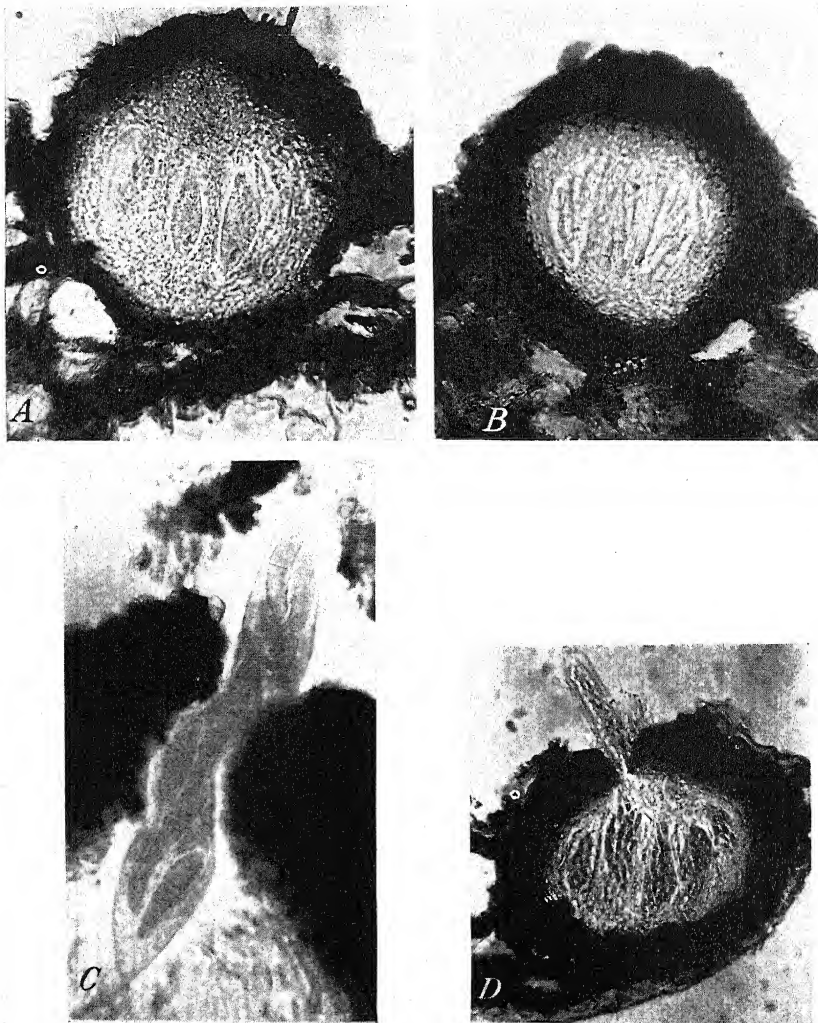
FIGURE 2.—*A*, Immature ascoma of *Rehmiellopsis balsameae*, showing developing asci ($\times$ about 320); *B*, immature ascoma of *R. balsameae* with young ascospores ($\times$ about 320); *C*, ascus with spores of *R. balsameae* ($\times$ about 600); and *D*, ascoma of *R. abietis*, showing asci and spores ($\times$ about 275).

and eventually the epidermal cells. Fragments of both kinds of cells are found within the young stroma for some time, but these gradually disintegrate. For the most part, the cells of the young fruiting body

turn a dark brown, leaving only a small central group of hyaline cells containing large oil globules. The hyaline cells develop into a pseudoparenchymatous tissue, which soon involves the greater part of the globoid stroma. A few of the outer cell layers of the stroma develop heavy brown walls, particularly just below the leaf cuticle, which, in August, is split by the rapidly developing fruiting body. The pseudoparenchymatous tissue becomes more loosely woven, and when the fruiting body is crushed on a slide this tissue is extruded as individual globoid, densely granular, hyaline cells that might be mistaken for spores. By late September the outer layers resemble a relatively thick perithecial wall, which merges into an inner layer of subhyaline tissue surrounding the pseudoparenchymatous tissue of the centrum.

In October the young asci become noticeable, developing slowly from a very limited, flat, basal hymenial layer (fig. 2, *A*). The central asci grow straight up through the stromatic tissue, but the surrounding asci curve outward from the hymenial layer, following the contour of the globoid body. As the thick-walled asci grow upward, the pseudoparenchymatous cells ahead of them break down and disappear, leaving very narrow compressed cells between the asci. In other words, the asci are always surrounded by the pseudoparenchymatous cells (fig. 2, *B*). When cross sections are cut through the needles, entire asci may drop out, leaving cavities completely surrounded by the stromatic tissue. No paraphyses are present. The fruiting body very frequently develops in the tissue at the edge of the needles and occupies the entire space between the two leaf surfaces. It always opens toward the upper surface, rupturing the cuticle by the formation of a small papillalike protrusion.

There is no further conspicuous development of the asci during the winter, but in early March the outlines of the spores are noticeable and the walls of the asci increase in thickness, particularly at the tips. By the time the spores are mature, the heavy wall-like layers of brown cells at the top of the fruiting body split apart near the center of the papillalike protrusion. No true ostiole is formed. Under favorable conditions of moisture, the pseudoparenchymatous tissue above and surrounding the asci gradually disintegrates and the spores are discharged. Only the dark wall-like layer of cells remains after the spores are extruded, but the cavity soon becomes filled with brown-walled cells forming a sclerotiumlike body. Needles bearing these bodies usually fall off during the following summer.

<div align="center">Asci</div>

The asci are extremely heavy-walled, particularly at the tips (figs. 2, *C*, and 3, *D*). The walls measure approximately 5μ to 8μ at the thickest part. The asci are clavate, straight or curved, adhere together in a fascicle, and measure 81μ to 141μ by 33μ to 41μ. They are multispored, containing 16 spores irregularly arranged, and aparaphysate but surrounded by pseudoparenchymatous tissue.

As the ascus matures, a slight internal rupture appears in the heavy wall near the apex and the epiplasmic content expands upward (fig. 4, *A*). This expansion continues, carrying the spores with it (fig. 4, *B*), until the top of the ascus is ruptured (fig. 4, *C*). The epiplasm and spores extrude in the form of a clavate endoascus (fig. 4, *D*), which eventually withdraws almost completely from the primary sheath

(fig. 4, *E*). The final stage in the liberation of the spores in nature has not been observed, but when mounted in water the entire epiplasmic structure of the mature endoascus collapses, freeing the spores. The latter are usually held together in a gelatinous mass for a short time after expulsion. A similar development of an endoascus has been reported in certain genera of the Sphaeriales, Dothideales, and Myriangiales (*4*), but the character of the ascus wall and the method of spore discharge vary in the different genera. In the fungus here described, the exact nature of the enveloping layer of the expanding endoascus could not be determined; but the layer is extremely thin, though resistant to pressure and moisture until the greater part of the epiplasm and most of the spores have been extruded from the primary
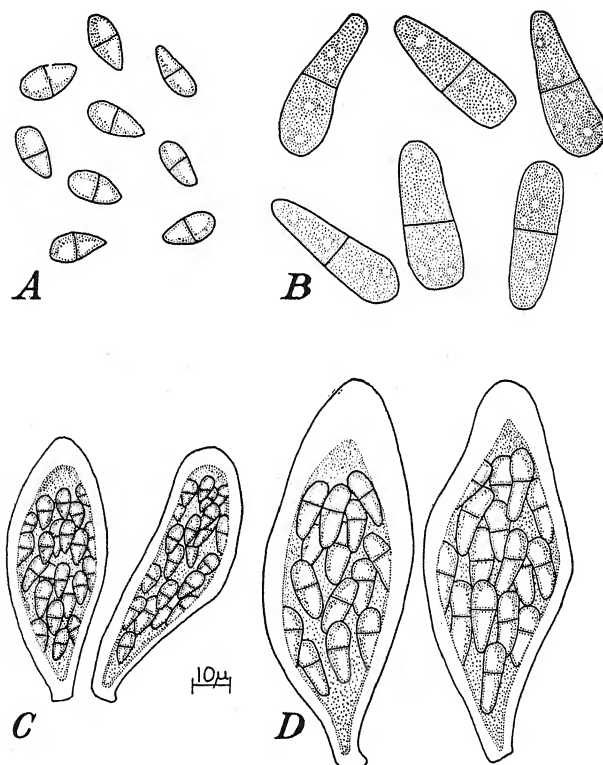
FIGURE 3.—*A*, Mature ascospores of *Rehmiellopsis abietis; B*, mature ascospores of *R. balsameae; C*, immature asci and spores of *R. abietis; D*, immature asci and spores of *R. balsameae*. Drawings made with the aid of a camera lucida.

wall or sheath. This suggests the interpretation given by Stevens and Weedon (*24*), namely, that the epiplasm holds the spores together, thus resembling an ascus.

ASCOSPORES

The ascospores (fig. 3, *B*) are ellipsoidal, two-celled, hyaline, densely granular, heavy-walled, and sometimes slightly constricted at the septum and slightly curved (fig. 2, *C*). The cells are unequal, the one toward the tip of the ascus being shorter, slightly wider, and

rounded at the tip, while the lower cell is more tapering but is also rounded at the end. The spore measurements are as follows: [6] From *Abies concolor* (25 spores), 31.5μ to 47.9μ by 6.3μ to 12.6μ; from *A. balsamea* (25 spores), 37.8μ to 49.9μ by 8.8μ to 12.6μ.

TAXONOMY

In order to determine the identity of the fungus causing the tip blight of species of *Abies* in the United States it was necessary to review the taxonomy of the species of *Rehmiellopsis* causing a similar disease in Europe. In 1905 E. Rostrup (*21*) described *Sphaerella abietis* as the cause of a disease of species of *Abies* in Denmark. He reported the asci as cylindric, 50μ by 10μ, and 8-spored, and the spores as bilocular, colorless, 12μ to 16μ by 5μ to 6μ, with the upper cell the
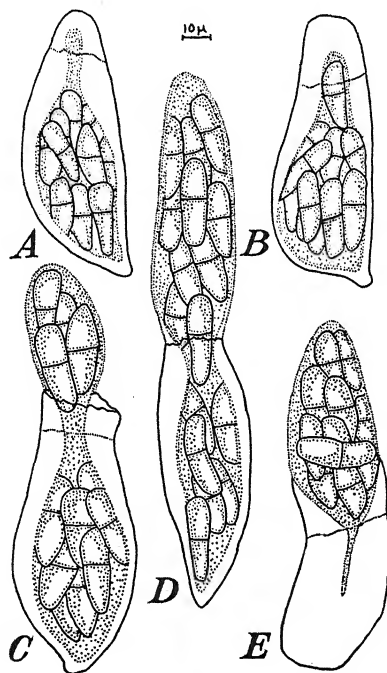
FIGURE 4.—Asci of *Rehmiellopsis balsameae*, showing extrusion of epiplasm and spores from the primary sheath: *A*, Internal rupture of sheath and expanding epiplasm; *B*, spore in expanding epiplasm; *C*, rupture of sheath and extrusion of epiplasm and spores; *D*, epiplasm and spores forming an endoascus; *E*, almost complete withdrawal of endoascus from primary sheath.

larger and with a constriction at the septum. In 1910 Bubák (*2*) also reported a disease of species of *Abies* occurring in Bohemia and caused by an ascomycete with fruiting bodies that opened irregularly and were composed of pseudoparenchymatous tissue. The asci contained 10 to 24 hyaline uniseptate spores. Bubák concluded that, on the

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[6] The spores from these two hosts, as well as those mentioned later from *Abies alba* and *A. lasiocarpa*, were all measured in the same manner. Mature fruiting bodies from herbarium material were crushed on slides in a solution made as follows: Potassium acetate, 10 gm.; pure glycerine, 200 cc.; 95-percent alcohol, 300 cc.; and distilled water, 500 cc. The mounts were allowed to stand in the solution for 24 hours, and 25 spores on each mount were then measured with a filar micrometer. To facilitate the measuring of the spores from *A. alba* and *A. lasiocarpa*, a stain (10 gm. of erythrosin) was added to the solution.

basis of these characters, the fungus did not correspond with any genus previously described; he therefore established the genus *Rehmiellopsis*, designating the species on *Abies* as *R. bohemica* Bub. and Kab. A few years later, O. Rostrup (*22*) found that *S. abietis* E. Rostr. also had more than 8 spores and corresponded in all respects with Bubák's *R. bohemica*. He therefore designated it as *R. abietis* (E. Rostr.) O. Rostr.

In the meantime Lindau (*16, p. 534*) had transferred E. Rostrup's *Sphaerella abietis* to the genus *Mycosphaerella* Johans., and Lind (*15, p. 204*) also published it as *M. abietis* (Rostr.) Lindau, with 8-spored asci. In Saccardo's description of *Mycosphaerella* (*23, v. 9, p. 659*) there is the statement that the genus was established by Johanson in 1884 to include the species formerly placed in the genus *Sphaerella* Fries, since that generic name was preoccupied by the algal genus *Sphaerella* Sommf. However, Saccardo retained the name *Sphaerella* for those species of the fungus genus that had 8-spored asci, and revised Johanson's description of *Mycosphaerella* to include only those species having 16 spores in the ascus. In 1928 he published Bubák's *Rehmiellopsis bohemica* as *M. bohemica* (Bub. and Kab.) Sacc., with *R. abietis* (Rostr.) Rostr. as a synonym (*23, v. 24, p. 893*).

In 1920 Von Höhnel (*11*) questioned the validity of the genus *Rehmiellopsis* and its relation to *Mycosphaerella*. He pointed out that Bubák had placed *M. polyspora* Johans. in the genus *Rehmiellopsis*, since it has more than 8 spores in an ascus, and had also described a new species, *R. conigena*, with 16-spored asci, occurring on cones of *Pinus halepensis* Mill. and *P. pinea* L. (*3*). The latter fungus was found by Von Höhnel (*10*) to be identical with *Sphaeria strobiligena* Desm., which had proved to be a dothideaceous fungus and which he believed to be correctly named *Hariotia strobiligena* by Karsten, as reported by Saccardo (*23, v. 9, p. 672*). Von Höhnel stated also that *R. bohemica* might be a dothideaceous fungus in the same genus.

The question therefore arises as to the proper classification of Rostrup's *Sphaerella abietis*, whether it is a sphaeriaceous fungus in the genus *Mycosphaerella* or a species of the dothideaceous genus *Hariotia*. The type specimen of *S. abietis* Rostr. was not available to the writer, but an examination of Bubák's material on which he based the genus *Rehmiellopsis* showed that the fruiting structure is a simple, globoid, stromatic body with an outer periderm of thick-walled cells resembling a perithecial wall. As Bubák pointed out (*2*), the interior consists of pseudoparenchymatous tissue in which the asci develop, and the fruiting body opens irregularly without the formation of an ostiole. The asci are liberated by the dissolution of the stromal cells directly above them. For the most part these characters correspond with those of the family Pseudosphaeriaceae established by Von Höhnel (*8, 9*) and with the order Pseudosphaeriales, of Theissen and Sydow (*25*). This order includes genera of the Sphaeriales and particularly of the Mycosphaerellaceae, in which the fructifications have no true perithecial wall and ostiole and are composed of pseudoparenchymatous tissue surrounding the developing asci. Petrak (*20, p. 67*), in a discussion of the Pseudosphaeriaceae, interpreted the strands of tissue between the asci as primitive paraphyses, resulting from the pressure of the developing asci, and called them paraphysoids. In Bubák's specimen, no paraphysoids of this

type are present but the asci are separated by narrow cells of the pseudoparenchymatous tissue. In a crushed mount of a mature fruiting body this tissue is extruded as individual cells and not as strands.

Miller (*17*) has indicated that the pseudosphaeriaceous type of ascocarp is similar to that of the Dothideales, in which the asci are surrounded with stromal tissue. He pointed out that the paraphysoids of the Pseudosphaeriales develop as definite threads from ascogenous hyphae and are not the result of compression of the tissue by the developing asci (*18*). He considered this one of the points of distinction between the Dothideales and the Pseudosphaeriales, the genera *Dothidea* and *Mycosphaerella*, which have no paraphyses or paraphysoids, being representative of the former order. However, most species of *Mycosphaerella* have been described as having ostiolate perithecia and are commonly classified in the Sphaeriales, although a few have been reported as dothideaceous (*6, 19*). Since Bubák's *Rehmiellopsis bohemica* and the species described in the present study are of the dothideaceous type, it seems inadvisable to consider them species of *Mycosphaerella* until further investigations of that genus have been made.

As already mentioned, Von Höhnel (*11*) considered it possible that Bubák's *Rehmiellopsis bohemica* might prove to be a dothideaceous fungus in the genus *Hariotia* Karsten. An examination of Desmazières' *Sphaeria strobiligena*, upon which Karsten based the genus *Hariotia* and which Von Höhnel stated was identical with *R. conigena* Bub., shows that the fruiting body is not at all the same as in *R. bohemica*. In the former the stroma is pulvinate rather than globoid, with a hypostroma, and is conspicuously erumpent, with the periderm differentiated but not resembling a perithecial wall; the asci are numerous and do not adhere in a fascicle. Therefore *R. bohemica* cannot be considered a species of *Hariotia*, nor does it correspond exactly with any other genus of the Dothideales.

Since Bubák's *Rehmiellopsis bohemica* does not correspond with *Mycosphaerella* in the Sphaeriales or with *Hariotia* in the Dothideales, it is at present difficult to assign the genus *Rehmiellopsis* Bubák to any definite place in the systematic key. A cytological study of *Rehmiellopsis* in comparison with similar dothideaceous genera and also with the species of *Mycosphaerella* having dothideaceous characters might reveal the relation of *Rehmiellopsis* to those genera. Under the circumstances, the writer has chosen to retain the generic name *Rehmiellopsis*, with Bubák's *R. bohemica* as a synonym of *R. abietis* (E. Rostr.) O. Rostr., and to assign the genus to the Dothideales.

A detailed study of specimens of *Rehmiellopsis abietis* from various sources indicated the important points of distinction between this species and the one occurring in the eastern part of the United States. Included in the study were the following specimens.

On *Abies alba:* Wartenberg, Bohemia, collected by J. E. Kabat, determined by F. Bubák, April 1909, type specimen, on file in herbarium of the Brooklyn Botanical Garden, Brooklyn, N. Y.; S. Hareskov, Denmark, coll. and det., O. Rostrup, Oct. 1925, not type material, submitted to the writer by G. D. Darker; Almindingen, Bornholm, Denmark, coll. and det., J. S. Boyce, Sept. 1925, No. 1546, herbarium of J. S. B.; Loch Awe, Argyllshire, Scotland, coll., J. S. Boyce, det., M. Wilson, Aug. 1925, Nos. 1547 and 1548, herbarium of J. S. B.

On *A. nobilis:* Corrour, Inverness-shire, Scotland, coll. and det., J. S. Boyce, Aug. 1925, No. 1549, herbarium of J. S. B. On *A. lasiocarpa:* Edgewood, British Columbia, coll., L. N. Goodding and J. W. Kimmey, June 1932 and Sept. 1935, det., A. M. Waterman, Forest Pathology collections 93326 and 93327.

The characteristics of the hyphae and the development of the ascomata are the same in both species. However, the mature ascomata of *Rehmiellopsis abietis* (fig. 2, *D*) are slightly smaller, containing a greater number of smaller asci with narrower walls and smaller spores. The asci measure 50μ to 90μ by 20μ to 22μ, with walls 3μ to 5μ at the thickest part, and contain 16 to 24 spores irregularly arranged (fig. 3, *C*). The spores (fig. 3, *A*) are 2-celled, hyaline, finely granular, with thin walls, not constricted at the septum, straight, with the 2 cells of about equal length, the upper cell very slightly the broader and the lower definitely acute at the tip. The spore measurements are as follows: From *Abies alba* (25 spores), 11.1μ to 21μ by 4μ to 6.3μ; from *A. lasiocarpa* (25 spores), 12.6μ to 18.5μ by 4.2μ to 6.7μ.

Bubák (*2*) reported a pycnidial fungus, in association with the perfect stage of *Rehmiellopsis abietis*, on *Abies alba* in Bohemia, and named it *Phoma bohemica*. He stated (*p. 318*) that "Es ist vollkommen sicher, dass beide Pilze genetisch verbunden sind," but gave no proof. The similarity in the early development and superficial appearance of the two types of fruiting bodies and the fact that the pycnidial form apparently was always associated with the perfect stage seemed to be the basis for the statement. E. Rostrup (*21*) reported a similar pycnidial fungus on infected *Abies* in Denmark, and O. Rostrup (*22*) also mentioned it on *A. alba*. Jørstad (*13*) was not certain that he found this imperfect stage in Norwegian material, but Wilson and MacDonald (*28*) reported it on species of *Abies* in Scotland. In the writer's examination of the herbarium specimens previously mentioned, this imperfect stage was found only on Bubák's specimens of *A. alba* and on those of *A. lasiocarpa* from British Columbia. The writer has not made a cultural study of this pycnidial fungus from fresh material to prove its relationship to *R. abietis*.

Bubák's (*2, p. 320*) description of these pycnidia reads as follows: "contextu crasso, nigrofusco, pseudoparenchymatico, intus paulatim hyalino, papilla conica erumpentibus, hicque irregulariter dehiscentibus." This suggests a method of development resembling that of the perfect stage, which is indeed the case. Bubák, however, reported the presence of conidiophores: "basidiis cylindricis, brevibus, ad apicem attenuatis, hyalinis vel parum luteolis"; but in fact the spores are produced directly from the cells of the hymenial layer, which are slightly modified in shape, suggesting "ad apicem attenuatis." While the spores are forming, the pseudoparenchymatous tissue of the interior of the fruiting body undergoes disintegration and the short papilla is ruptured without the formation of an ostiole. It is evident that the fungus is not a true *Phoma* or *Macrophoma*, but according to the classification of imperfect fungi given by Clements and Shear (*5, p. 178*), it might be considered a species of *Dothichiza*. The spores in the *Abies lasiocarpa* material corresponded in all respects with those found in Bubák's specimens. No imperfect fungus of this type has been found in the many collections of the species of *Rehmiellopsis* on *A. balsamea* and *A. concolor* examined by the writer.

The species of *Rehmiellopsis* on *Abies balsamea* and *A. concolor* in the eastern part of the United States is designated as *R. balsameae* n. sp., on the basis of the size of asci and spores and the smaller number of spores per ascus. For the sake of comparison the two species of *Rehmiellopsis* on species of fir are here described.

Rehmiellopsis abietis (E. Rostr.) O. Rostr. (emended description).
    Syn.: *Sphaerella abietis* E. Rostr., 1905, Tidsskr. Skov. 17: 39.
            Mycosphaerella abietis (E. Rostr.) Lind., 1908, *in* Sorauer: Handb. Pflanzenkr. Aufl. 3, Bd. 2: 534.
            Rehmiellopsis bohemica Bub. and Kab., 1910, Naturw. Ztschr. f. Forst. u. Landw. 8: 320.
            Mycosphaerella bohemica (Bub. and Kab.) Sacc., 1928, Syll. Fung. 24: 893.

Ascomata amphigenous, usually epiphyllous, subepidermal becoming erumpent, single or rarely aggregate, globose, pseudoparenchymatous with differentiated periderm on all sides, opaque at the top, papillalike, rupturing irregularly, 150μ to 200μ in diameter. Asci clavate to cylindric, short stipitate, thick-walled, thickened at the apex, 3μ to 5μ at thickest point, straight or slightly curved, adhering together in a fascicle, 50μ to 90μ by 20μ to 22μ, many-spored (16 to 24), no paraphyses. Ascospores ellipsoid, irregularly arranged, hyaline, 11.1μ to 21μ by 4μ to 6.7μ, uniseptate, not constricted at septum, finely granular, straight, cells about equal in length, upper cell slightly broader and rounded at tip, lower cell tapering with acute point.

Pycnidia subepidermal becoming erumpent, single, globose with slight papilla, opening irregularly, pseudoparenchymatous, 150μ to 200μ in diameter. Spores oblong or fusiform, straight or slightly curved, slightly pointed at both ends, hyaline, continuous, 10μ to 16μ by 4μ to 6.5μ, produced directly from the cells of the hymenial layer.

Habitat.—In Europe: On living leaves of *Abies alba* in Denmark, Norway, Bohemia, and Scotland; of *A. pinsapo* Boiss. in Denmark, Norway, and Scotland; of *A. nobilis* and *A. cephalonica* in Denmark and Scotland; of *A. nordmanniana* (Steven) Spach in Denmark; of *A. sibirica* Ledeb. in Norway; and of *A. pindrow* Royle in Scotland. In North America: On living leaves of *A. lasiocarpa* in British Columbia, Canada.

Rehmiellopsis balsameae n. sp.

Ascomata amphigenous, usually epiphyllous, subepidermal becoming erumpent, single or rarely aggregate, globose, black, pseudoparenchymatous with differentiated periderm on all sides, opaque at the top, papillalike, rupturing irregularly, 200μ to 250μ in diameter. Asci clavate to cylindric, short stipitate, thick-walled, conspicuously thickened at the apex, 5μ to 8μ at thickest point, straight or curved, adhering in a fascicle, 81μ to 141μ by 33μ to 41μ, 16-spored, no paraphyses. Ascospores fusiform-elliptic, irregularly arranged, hyaline, 31.5μ to 49.9μ by 6.3μ to 12.6μ, 1-septate, sometimes slightly constricted at septum, densely granular, straight or curved, cells unequal, upper cell shorter and slightly broader, lower cell longer and tapering but rounded at end.

Conidial stage not observed.

Ascomatibus amphigenis sed plerumque epiphyllis, subepidermicis, innato-erumpentibus, sparsis vel rare aggregatis, globosis, nigris, contextu pseudo-parenchymatico, astomis, 200μ–250μ in diam.; ascis cylindraceo-clavatis, breve stipitatis, membrana crassa, 5μ–8μ ad apicem, rectis vel curvatis, 81μ–141μ× 33μ–41μ, 16-sporis, aparaphysatis; sporidiis fusiformi-ellipticis, inordinatis, hyalinis, 31.5μ–49.9μ×6.3μ–12.6μ, 1-septatis, interdum leniter constrictis, rectis vel curvatis, cellulis plerumque inaequalibus, utrinque obtusis.

Fructificationibus conidicis non visis.

Habitat.—On living leaves of *Abies concolor, A. balsamea, A. cephalonica, A. nobilis*, and *A. fraseri.* Type specimen, 93300,[7] on leaves of *A. concolor*, Hamilton, Mass., collected by M. A. McKenzie and K. F. Aldrich, May 4, 1934, has been deposited in the Mycological Collections, Plant Industry Station, Beltsville, Md. Cotype specimens are filed in the herbaria of the New York Botanical Garden and the Brooklyn Botanic Garden and in the Farlow Herbarium, Harvard University, Cambridge, Mass. Additional specimens of the fungus on species of *Abies*, included in the study, are on file in the Forest Pathology Laboratory, Bureau of Plant Industry, Soils, and Agricultural Engineering, at New Haven, Conn., as follows:

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[7] Collection numbers are those of the Division of Forest Pathology.

On Abies concolor.—Massachusetts: 93301, Hamilton, coll. A. M. Waterman and K. F. Aldrich;[8] 93302, Hamilton, coll. K. F. A. Maine: 93303, Augusta, coll. K. F. A. and M. A. McKenzie; 93304, Augusta, coll. K. F. A.; 93305, Cape Elizabeth, coll. R. W. Nash; 93312, Flagstaff, coll. K. F. A. New Hampshire: 93308, Rye Beach, coll. Mrs. James Morrison. New York: 93306, Lake George, coll. K. F. A. and McK.; 93307, Lake George.

On Abies balsamea.—Maine: 88963, Eustis, coll. J. R. Hansbrough; 94184, Flagstaff; 93313, South China, coll. C. K. Goodling; 93314, Ripogenus Dam; 93315, Oquossoc; 93316, Jim Pond Township; 93317, Kokadjo; 93318, Pittston Farm; 93319, Sandwich Township. New Hampshire: 93320, Stewartstown; 93321, Colebrook; 93322, Errol; 93323, Second Connecticut Lake; 93324, Pittsburg. Vermont: 93325, Lemington.

On Abies cephalonica.—Rhode Island: 93309, Bristol.

On Abies nobilis.—Massachusetts: 93310, Hamilton.

On Abies fraseri.—Massachusetts: 93311, Hamilton.

CULTURAL CHARACTERISTICS

Cultures of *Rehmiellopsis balsameae* from single ascospores, asci, and newly infected leaf tissue were made on corn-meal, oatmeal, and potato agars, corn mush, potato plugs, and Leonian's (*14*) synthetic agar. The last-named gave the most uniform results. The ascospores germinated readily in water, even while still in the ascus, but hyphal growth on all the media was extremely slow. In about 48 hours the very short, brownish hyphae produced, on Leonian's agar, small, ovoid, hyaline spores, budding directly from the hyphal cells. This process continued for a few days only, giving a brownish-black yeast-like appearance to the agar. In the meantime a very limited amount of a reddish-brown aerial mycelium began to appear. This continued until a maximum growth of about 10 to 12 mm. in diameter was reached, after which there was no further development. On the other media growth was even more limited, consisting only of a slight yeastlike growth followed by an exceedingly scanty aerial growth.

Transfers were made from single-ascospore cultures on Leonian's medium to sterilized potato plugs and to sterilized needles of *Abies concolor*. On the former, only a yeastlike growth, with a very little aerial mycelium, developed. On the sterilized needles, immature fruiting bodies characteristic of *Rehmiellopsis* were formed in about 2 weeks. These fruiting bodies did not produce spores of any kind, but their method of development was identical with that found in nature. The mycelium produced in cultures on Leonian's medium and in the sterilized needles was comparable in color and width of hyphae with that found in the leaf tissues in nature when fruiting bodies are producing spores. Cultures, both on agar and on sterilized needles, were grown in the greenhouse to determine whether conditions of light and heat different from those in the laboratory might influence the development of mycelium and fruiting bodies. No change of any kind, however, was evident. Single-ascospore cultures from *A. balsamea*, grown on Leonian's medium, were identical with those of the fungus isolated from *A. concolor*.

PATHOGENICITY

PATHOLOGICAL ANATOMY

In the needles of both *Abies balsamea* and *A. concolor* the hyphae of *Rehmiellopsis balsameae* are usually evident first in the intercellular spaces below the stomata. The cells of the mesophyll in the vicinity

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[8] Unless otherwise indicated, collections were made by A. M. Waterman and K. F. Aldrich.

soon turn brown, and the hyphae branch out between them. The browning of the cells very conspicuously precedes the advance of the mycelium, suggesting the possibility of a toxic effect by the fungus. A similar condition was reported by Wilson and MacDonald (*28*) in their examination of the effect of *R. abietis* on *A. alba* in Scotland. As the browning continues, the leaf cells become shrunken and gradually filled with mycelium until all the tissue, except the vascular cells, is affected. The vascular tissue shows no invasion by the hyphae until the complete destruction of all other leaf tissue. The spread of the mycelium in the leaf tissue is relatively slow, and the massing of the hyphae for the formation of fruiting bodies is usually evident before the destruction of all the cells in the area.

In some cases on *Abies concolor*, the mycelium from the leaf penetrates into the twig, causing a small canker around the base of the leaf. This seems to occur if infection takes place before the leaf reaches maturity or if the locus of infection is at or near the base of the leaf. The mycelium spreads into the twig first at the periphery of the absciss layer, and in some cases it does not penetrate farther. If the vascular tissue is affected, however, the browning of the twig cells occurs slowly and is followed by the spread of the hyphae, resulting in the formation of a canker. Cankers are formed on the terminal twigs more frequently than on laterals, but both terminal and lateral twigs may be killed back partly or entirely to the nodes. This dieback occurs when infection takes place in the majority of the needles of a twig before they reach maturity, and every needle is eventually affected. All the cells of the twig become brown and shrunken and even the cells of the absciss layer are invaded by the fungus. The death of the twigs, therefore, seems to be the result of the accumulated effects of the fungus in all the needles, whereas the formation of a canker depends on the spread of the mycelium from one infected needle. The partial dieback of a twig is followed by the development of adventitious buds below the lesion, but the growth developing from such buds is usually weak and stunted. No cankers have been found on *A. balsamea*, but both terminal and lateral twigs frequently die back to the node.

The effect of the disease on the tree as a whole depends upon the number of buds that escape infection and are able to produce a normal healthy growth the following season. When twigs are killed back to the node, further growth of that particular section of the branch is possible only from the adventitious buds. Since it is usually the laterals that are killed back, the continuance of growth from the normal terminal buds helps to counteract the effect of the dieback and is responsible for deferring the death of a diseased tree.

ARTIFICIAL INOCULATIONS

MATERIALS AND METHODS

Four series of inoculations were made in the greenhouse on 5-year-old potted trees of the following species of fir: *Abies concolor*, *A. fraseri*, *A. lasiocarpa* var. *arizonica* (Merriam) Lemm., *A. veitchii* Lindl., *A. homolepis* Sieb. and Zucc., and *A. holophylla* Maxim. In three of these series, the inoculum consisted of small amounts of mycelium and agar from single-ascospore cultures of *Rehmiellopsis balsameae*, isolated from *A. concolor* and grown on Leonian's (*14*) medium, and also aerial

mycelium from transfers of these cultures to sterilized needles of *A. concolor*. The mycelium was placed on buds or newly developed needles, and in two of the series the inoculated parts were wrapped in moist cotton and covered with a celluloid cylinder plugged at both ends with moist cotton. On the inoculated trees of the third series, this type of wrapping was not used but the trees were covered with bell jars shaded from the sun. In all cases, the coverings were removed in a week or 10 days and the trees were shaded from direct sunlight by cheesecloth. In the fourth series in the greenhouse, the inoculum consisted of a water suspension of crushed needles bearing fruiting bodies with mature asci and spores of *R. balsameae*, freshly collected from infected trees of *A. concolor*. By means of a pipette a few drops of the suspension were placed on the newly developing needles, and the whole shoot was then wrapped in moist cotton and a celluloid cylinder. The coverings were removed after the same interval as in the other series.

A nursery plot of 5- to 8-year-old trees of *Abies concolor, A. balsamea, A. fraseri, A. lasiocarpa* var. *arizonica, A. veitchii, A. nobilis, A. homolepis,* and *A. holophylla* was established for inoculations in a situation in Rhode Island that proved to be exceptionally favorable for the growth of most of the trees. *A. balsamea, A. lasiocarpa* var. *arizonica,* and *A. nobilis* were in general less vigorous but produced new growth sufficient for inoculations. A total of 189 inoculations were made in the following years: In 1935, 9 in May, 20 in July; in 1936, 14 in June; in 1938, 26 in June, 54 in July; in 1940, 66 in May. In the May 1935 series, 3 inoculations on 3 trees of *A. concolor* were made, the inoculum consisting of small pieces of mycelium and agar from single ascospore cultures of *Rehmiellopsis balsameae* isolated from *A. concolor*. The inoculum was placed on the upper surface of the newly developed needles of a terminal twig, and this twig and the two adjacent laterals were wrapped together in moist cotton and covered with a strip of waxed paper tied firmly at both ends. It was necessary to include the laterals, since the weight of the wrappings could not be supported by the tender terminal twig alone. In all the other series the inoculum consisted of infected needles of *A. concolor* or *A. balsamea* bearing mature fruiting bodies and spores of *R. balsameae* which had been slightly crushed in water to partly liberate the asci and spores. With sterilized forceps 3 or 4 of these crushed needles were placed on the upper surface of the newly developed needles of a terminal twig and wrapped as already described. In all cases the coverings were removed after an interval of 10 days or 2 weeks. Usually 2 or more inoculations were made on 1 tree, on widely separated shoots.

In the spring of 1941 an attempt was made to devise some method that would be more nearly comparable to the conditions under which infection takes place in nature and would eliminate the cotton and waxed-paper wrappings. "Iceless refrigerators" of the type described by Hunt (*12*) were built with a framework 2 feet wide by 2 feet deep by 4 feet high and were placed over two trees each of *Abies concolor* and *A. balsamea*. Twigs of these same species of *Abies,* bearing infected needles with mature fruiting bodies and spores of *Rehmiellopsis balsameae,* were kept moist for about 12 hours after collection and then fastened firmly on the young shoots of the current season's growth by means of fine copper wire wrapped around the

twigs of the previous year's growth. Five cross inoculations were made on each tree, and the infected needles were carefully intermingled with the needles of the young shoots. Pans 6 inches deep were then set on the framework and filled with water. The whole framework was covered with two thicknesses of unbleached cotton cloth, which was constantly moistened with the water from the pans. During bright sunlight in the middle of the day the cloth was kept thoroughly soaked with additional water from a garden hose. The iceless refrigerators were removed after 48 hours, but the twigs and needles used as inoculum were left wired to the trees for 2 weeks.

Experimental Results

Two of the series of inoculations in the greenhouse were made in late February and early March, before the buds had opened, to determine whether bud infection was responsible for the earliest symptoms of the disease in nature. No infection occurred in any case, and the fungus seemed to be unable to penetrate the bud scales or to infect the twig tissue at the base of the buds. Later in the season, when the new needles were emerging from the buds, the other series were made, but no infection resulted. It was thought that the greenhouse conditions, particularly temperature, were unfavorable for the fungus.

In the first series of inoculations in the nursery plot in May 1935, 3 inoculations on 3 trees of *Abies concolor*, made with mycelium of *Rehmiellopsis balsameae* from culture as inoculum, resulted in infection on 2 of the trees. The following October a few of the needles showed fruiting bodies, and upon examination these were found to contain developing asci characteristic of *R. balsameae*. The fungus was not reisolated at this time, since the remaining infected needles were left on the trees to overwinter to determine whether mature spores would be produced in the spring. However, none of the infected needles could be found in the spring of 1936, having broken off during the winter. Of the 26 inoculations made in 1935 with crushed infected needles of *A. concolor* as inoculum, 7 were made on *A. concolor*, 6 on *A. fraseri*, 6 on *A. holophylla*, 3 on *A. lasiocarpa* var. *arizonica*, 2 on *A. homolepis*, and 2 on *A. veitchii*. No positive results were obtained.

In 1936 the type of inoculum just described was used in the 14 inoculations, as follows: 4 on *Abies concolor*, 6 on *A. fraseri*, 1 on *A. holophylla*, 2 on *A. homolepis*, and 1 on *A. veitchii*. In October immature fruiting bodies were found in all 4 of the inoculations on *A. concolor* and in 2 of those on *A. fraseri*. No material was collected for reisolating the fungus, but all infected needles were left to overwinter on the trees. The following spring 1 inoculation on each of these 2 species of *Abies* had resulted in the formation of mature fruiting bodies and spores of *Rehmiellopsis balsameae*. Because of the limited amount of infected material, no attempt was made to reisolate the fungus. All other needles that had produced fruiting bodies in the autumn had fallen off during the winter. No infection occurred on the 3 other species of *Abies*.

In 1938 and 1940 further attempts were made to obtain infection by cross inoculations with the same type of inoculum as was used in 1936 and to carry infection through to the following spring, thus obtaining

mature spores and, if possible, natural infection of the new growth. Table 1 shows the number of inoculations made in the 2 years. The phrase "possible infections" in the table indicates the presence of characteristic symptoms of infection and what appeared to be fruiting bodies of *Rehmiellopsis balsameae* as seen under a hand lens when examined in the autumn. None of the diseased needles were removed at that time and no reisolation of the fungus was attempted, because of the very limited number of needles showing infection and the lack of sufficiently distinctive cultural characteristics for an exact identification. During the winter and early spring in both years, however, all apparently infected needles were broken off by winds or storms and no evidence of the fungus could be found. Therefore the results of these inoculations are entirely inconclusive.

It was also found that several days of rain during the period when the inoculated shoots were covered with cotton and waxed-paper wrappings would cause a serious mold of both needles and twigs, even in the controls. In some cases the entire shoots would break off when the wrappings were removed, and it was evident that all shoots were appreciably weakened by the process. For this reason it was thought that the iceless refrigerators would prove more efficient. However, in the 20 cross inoculations made under these conditions no positive results were obtained. On 3 of the inoculated shoots of *Abies balsamea* a few apparently infected needles were found the following autumn, but by the next spring these needles had broken off.

In the inoculations made by artificial methods, therefore, positive results were obtained from three inoculations on *Abies concolor* and one on *A. fraseri*.

TABLE 1.—*Record of inoculations made on species of Abies with Rehmiellopsis balsameae*

Year and species of *Abies* inoculated	Source of inoculum			
	Abies concolor		*Abies balsamea*	
	Inoculations	Possible infections [1]	Inoculations	Possible infections [1]
1938	Number	Number	Number	Number
A. concolor	6	1	6	6
A. balsamea	12	0	12	8
A. fraseri	12	0	12	6
A. veitchii	2	0	4	1
A. nobilis	3	0	4	4
A. holophylla	3	0	4	1
Total	38	1	42	26
1940				
A. concolor	20	17		
A. balsamea	20	2		
A. fraseri	18	4		
A. veitchii	8	2		
Total	66	25		

[1] Characteristic symptoms and fruiting bodies, but no mature asci or spores. The needles broke off during the winter; therefore, the fungus did not reach maturity.

NATURAL INFECTION

In view of the difficulties encountered in obtaining positive results from cross inoculations by artificial methods, it was thought advisable

to test the susceptibility of various species of fir under natural conditions of infection. The results obtained from this experiment with seven species of *Abies* have already been reported (*26*), proving the susceptibility of *A. fraseri* and *A. nobilis* to *Rehmiellopsis balsameae*. *A. holophylla* was reported as apparently resistant, but on one of the trees of this species a few discolored needles were found in the fall of 1937. The next spring an examination of these needles showed mature fruiting bodies and spores of *R. balsameae*. It is evident that this species is only slightly susceptible and that the disease probably never would be of any significance on this host.

Mention has been made of the fact that surveys of infection areas in New England and New York have shown no indication of any connection between infection on native *Abies balsamea* and on introduced *A. concolor*. In order to test the possibility of cross infection of these hosts in nature, two small 6-year-old nursery trees of *A. concolor* were planted early in the spring of 1940 under the infected branches of two widely separated trees of *A. balsamea* growing in native stands in Flagstaff, Maine. Neither of the white firs showed evidence of infection that season, possibly because the season was unfavorable for infection even on native trees. Since the disease seemed to have subsided entirely on one of the balsam firs, the white fir in that vicinity was removed in the autumn. On the other white fir, symptoms of infection appeared in 1941, and in 1942 considerable infection was present on the newly developing growth (fig. 1, *C*), with mature fruiting bodies and spores of *Rehmiellopsis balsameae* on the 1941 needles. This, together with the previously reported results (*26*), gives definite proof that *R. balsameae* will cross-infect at least four species of fir: From *A. balsamea* to *A. concolor;* and from *A. concolor* to *A. fraseri, A. nobilis,* and *A. holophylla.* In this connection it is interesting to note that Boyce (*1*), in his observations of the disease of firs caused by *R. abietis* in Europe, pointed out that *A. nobilis* was the only American species of fir susceptible to that disease.

CONTROL

Since the tip blight on native balsam fir already is distributed over a large area of northern Maine, control measures for these forest trees would be impossible. The severity of the disease varies considerably from one season to another, and the affected trees show a striking ability to counteract the injury from infection, although some weakening always results after a fairly severe attack. For this reason, the tip blight has not yet caused any appreciable damage to older trees, but its particular menace in this region is to the young seedling trees. It is not known how long the disease has been prevalent on the native trees in Maine, but probably at least since 1930. Apparently tip blight has been moderately abundant across the border in Canada since 1919 (*7*). Its significance as a forest-tree disease in the United States is not yet definitely known.

Tip blight on white firs in the East is important because these trees are widely used as ornamentals and are highly valued for that purpose. Ornamental plantings of this species have been found as far north in Maine as Farmington, in the western part of the State, and Millinocket, in the eastern part. It is evident, from the results of the experiments with natural infection, that the planting of this species in the vicinity of infected balsam firs should be avoided.

Two series of spray experiments were undertaken to determine the possibility of controlling the disease on ornamentals by this means. In the infected planting of *Abies concolor* in eastern Massachusetts, 2 plots were selected in which the trees showed various degrees of infection. In 1 plot of 19 trees about 6 to 20 feet in height, 1 tree was heavily infected, 4 were slightly infected, and the remainder had only a few needles with fruiting bodies. A total of 26 other trees in the vicinity, lightly infected or free from disease, were left as checks. The second plot of 22 trees, which were of approximately the same height as those in the first plot and about half of which were heavily infected, was selected in the large planting where the disease was first found.

About the middle of May 1936, the first application of spray was made in both plots. The buds were then just opening, and the green of the new leaves was beginning to show. A 2–2–50 bordeaux mixture, with casein as a spreader, was applied thoroughly. A good coverage of the new growth was obtained, and no burning of the young needles resulted. A week later a severe frost injured the new growth of some of the trees in both plots. Only those needles at a certain stage of development, that is, just showing green in the bud, were affected, and the growth that had developed sufficiently to receive the application of spray was largely uninjured. A slight amount of infection, however, was becoming noticeable when the second application of spray was made, about 10 days after the first. The strength of the spray in this application was increased to 4–4–50. Some evidence of control was noticeable on the sprayed trees in comparison with the unsprayed, but no further application was made that season. A moderate amount of infection occurred on the sprayed trees after the second application, and on the unsprayed trees the infection was relatively heavy.

In 1937 the experiment was repeated in both plots, the 4–4–50 bordeaux mixture with casein being used for the first application. The spray was applied about a week later than in the preceding year, owing to a slightly later seasonal development of growth. Twelve days after the first application a second application of the same strength was made in the two plots, at which time no appreciable amount of infection was noticeable, even on unsprayed trees. In a week, however, a fairly severe attack of the disease occurred, and the contrast between sprayed and unsprayed trees was striking. The former showed only scattered needle infections, whereas on the latter much of the new growth was killed back to the nodes and needle infection was abundant. A third application of the spray was made 12 days after the second to protect the late-developing growth. The sprayed trees came through the season in fine condition and gave excellent evidence of the value of the treatment, even in areas of severe infection. No burning of the new growth resulted in any case, and good coverage as well as effective adhesiveness was obtained. There was no frost injury during the experiment.

From these experiments it was evident that the time element, particularly for the first application of spray, is of special importance in securing satisfactory results. The first application should be made as soon as the buds begin to show green and should be followed by two additional applications at intervals of about 10 or 12 days. The period during which infection takes place is comparatively short,

depending upon the stage of development of the new growth, the maturity of the fungus spores, and weather favorable for the germination of the spores. The height of severity of infection may fall within the period between the first two applications or between the last two, but the three applications should provide satisfactory control.

Bubák (2) and Wilson and MacDonald (28) advocated cutting off and burning branches of ornamentals affected by *Rehmiellopsis abietis* as soon as the disease appears, but this practice seems unnecessary for the control of *R. balsameae*. In fact, it might even retard the recovery of affected trees, since it is difficult to determine superficially what buds are affected, and healthy buds that would develop normal growth might be inadvertently removed. If the disease progresses on a tree for several seasons to the point where the lower branches are materially weakened or killed, these branches should be removed and burned for the sake of the general appearance of the tree. A carefully followed spray schedule, however, should prevent the killing back of the branches to any appreciable extent.

SUMMARY

A tip blight of native trees of *Abies balsamea* in northern New England and of ornamental trees of *A. cephalonica* in southern New England and *A. concolor* in New England and New York is caused by *Rehmiellopsis balsameae*, a new species. The disease in many respects resembles that caused by *R. abietis* in Europe on *A. alba, A. nobilis, A. pinsapo, A. nordmanniana, A. pindrow, A. cephalonica,* and *A. sibirica,* and in British Columbia on *A. lasiocarpa.*

The needles of the current season's growth are attacked by the disease, and a dieback of terminal or lateral shoots may result. Cankers are sometimes formed on twigs of *Abies concolor* at the base of infected needles.

Infection takes place early in the spring just as the new growth is developing. The fruiting bodies of *Rehmiellopsis balsameae* begin to form soon after infection, but the spores do not mature until the following spring. No imperfect stage of *R. balsameae* has been observed.

Inoculation experiments proved the pathogenicity of *Rehmiellopsis balsameae* on *Abies concolor* and also showed that *A. fraseri* is susceptible. Small nursery trees of *A. fraseri, A. nobilis,* and *A. holophylla,* planted in an area of infected trees of *A. concolor,* proved susceptible to natural infection. Cross infection from *A. balsamea* to *A. concolor* was obtained by planting a young tree of the latter species under a heavily infected native balsam fir.

The importance of the tip blight as a disease of forest trees is not yet definitely known, but because of its wide distribution on native balsam firs control measures would not be feasible in the forest. On ornamental white firs satisfactory control was obtained by three applications of spray at intervals of 12 days, of a 4–4–50 bordeaux mixture to which casein was added as a spreader. The first application of the spray should be made as soon as the new growth begins to emerge from the buds.

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EFFECT OF DIFFERENT SOIL COLLOIDS ON THE TOXICITY OF BORIC ACID TO FOXTAIL MILLET AND WHEAT[1]

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INTRODUCTION

Field experiments indicate that the toxicity of soluble boron compounds varies somewhat with the character of the soil. Whether the variation is due to different degrees of boron fixation by different soil colloids or to other properties of the soil has not been shown by experiments with plants. Laboratory experiments, however, indicate that soils have slight if any capacity for fixing borate ions in a form unavailable to plants, since practically all the borax added to a soil sample can usually be removed by leaching a few times with water. Instances have been reported of soils retaining added boric acid, but it is questionable whether the retained boric acid is unavailable to plants.

The experiments reported here were designed to show whether the colloidal material of a soil has a specific capacity for rendering borate ions unavailable to foxtail millet (*Setaria italica* (L.) Beauv.) and Marquis wheat (*Triticum aestivum* L.).

MATERIAL AND METHODS

The methods used were the same as those followed in studies with arsenates,[2] selenates,[3] and selenites.[4] Millet or wheat was grown in quartz-sand cultures and in sand-soil mixtures that contained sufficient soil to supply approximately 1 percent of colloidal material to the mixture. The effects of boric acid on growth of the plants in the two mediums were compared. If significantly more boric acid should be required to reduce growth by one-half in the sand-soil mixture than in the pure sand, the difference would be attributed to the colloidal material.

The comparison was based on the quantity of boron required to reduce growth one-half rather than on that required for maximum growth. The half-injury quantity, being much larger, could be determined more accurately. In fact, it was impossible to determine the boron required for the maximum growth of millet, since the plants evidently obtained all the boron they needed from impurities present in the materials employed.

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[1] Received for publication July 27, 1943.

[2] GILE, P. L. THE EFFECT OF DIFFERENT COLLOIDAL SOIL MATERIALS ON THE TOXICITY OF CALCIUM ARSENATE TO MILLET. Jour. Agr. Res. 52: 477–491, illus. 1936.

[3] LAKIN, H. W., and BYERS, H. G. EFFECT OF DIFFERENT SOIL COLLOIDS AND WHOLE SOILS ON THE TOXICITY OF SODIUM SELENATE TO MILLET. Jour. Agr. Res. 57: 1–20, illus. 1938.

[4] ——— and LAKIN, H. W. EFFECT OF DIFFERENT SOIL COLLOIDS ON THE TOXICITY OF SODIUM SELENITE TO MILLET. Jour. Agr. Res. 63: 559–581. 1941.

The experiments were conducted in glazed earthenware 1-gallon pots holding about 5 kg. of sand. The sand was maintained at a 15-percent water content by the addition of distilled water as required. Ten millet plants per pot were grown for periods ranging from 25 to 37 days, according to the season. At the end of the period either the heads were starting to appear or the nutrient salts were becoming depleted. Wheat was grown, five plants to the pot, for about 40 days; and dwarf sunflowers (*Helianthus* sp.), three plants to the pot, were grown for 35 days, when the buds appeared.

Except as otherwise noted, the following quantities of nutrient salts were added to each pot: Potassium nitrate, 0.93 gm.; ammonium sulfate, 0.33 gm.; magnesium chloride ($MgCl_2.6H_2O$), 0.42 gm.; sodium chloride, 0.05 gm.; ferric tartrate, 0.0185 gm.; manganese sulfate ($MnSO_4.2H_2O$), 0.0015 gm.; copper sulfate ($CuSO_4.5H_2O$), 0.00047 gm.; zinc chloride, 0.00013 gm.; and boric acid, 0.0011 gm. The phosphate applied varied with the estimated capacities of the soils for rendering phosphate unavailable. The pure quartz sand and the Colby, Conowingo, and Muskingum soil-sand mixtures received 0.36 gm. of monocalcium phosphate ($Ca(H_2PO_4)_2.H_2O$) per pot; the Spearfish and Manor soil-sand mixtures 0.54 gm.; and the Nacogdoches, Kirvin, A horizon, and Kirvin, B horizon, soil-sand mixtures, 0.72 gm. of phosphate per pot. All these salts, together with the differential applications of boric acid, were added in solution to the top of the pot before the seed was sown.

The soils used are designated here by their series names. The type names of the soils, the depth of the samples, and the laboratory numbers are given in a previous publication,[5] which also gives references to other publications in which detailed data may be found on the chemical and mechanical composition of the soils. The Conowingo soil is described in another publication.[6]

EXPERIMENTAL RESULTS

A preliminary experiment was made to determine approximately the quantity of boric acid required to reduce the growth of millet in quartz sand by one-half. The results are shown in table 1.

In this experiment the 0.0002 gm. of boron present in the usual basic fertilizer was omitted, but the application of 0.0001 gm. of boron did not increase the yield. This is in agreement with unpublished tests in which 0.0005 gm. of boron per pot likewise failed to increase the yield of millet in quartz sand. Evidently impurities in the materials used supplied all the boron needed. The results of this experiment indicate that the quantity of boron required to reduce growth one-half is much greater than the quantity required for maximum growth.

A second preliminary experiment was conducted with dwarf sunflowers, to determine whether boron impurities were sufficient to supply a plant with a high boron requirement. In this experiment, also, boron was omitted from the basic fertilizer. The results are shown in table 1.

[5] See footnote 4, p. 339.
[6] ROBINSON, W. O., EDGINGTON, G., and BYERS, H. G. CHEMICAL STUDIES OF INFERTILE SOILS DERIVED FROM ROCKS HIGH IN MAGNESIUM AND GENERALLY HIGH IN CHROMIUM AND NICKEL. U. S. Dept. Agr. Tech. Bul. 471, 29 pp. 1935.

Agreement between replicated pots was poor in this experiment, since only 3 plants were grown per pot, but the average yields form a regular curve when plotted against the boron applications except for the point representing the application of 0.0008 gm. The average yields are evidently sufficiently accurate to show that the sunflowers needed about 0.001 gm. of boron per pot for their maximum growth in the sand cultures and that something like 10 times as much boron was needed to reduce growth by one-half. It may be concluded that boron impurities in the materials used were too small to affect the half-toxicity values appreciably.

TABLE 1.—*Effect of boric acid on millet and dwarf sunflowers in quartz sand*

EXPERIMENT 1 (MILLET; SEPT. 28 TO OCT. 28, 1940)

Boron added per pot	Air-dry yield of individual pots					Average air-dry yield per pot	Boron per pot required to reduce yield by one-half
Gram	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*
			2.25	2.68	2.38	2.44	
.0001			2.37	2.20	2.75	2.44	
.0050			2.21	2.27	2.34	2.27	0.016
.0100			1.88	2.14	1.90	1.97	
.0200			.93	.97	.30	.73	
.0400			.06	.25	.03	.11	

EXPERIMENT 2 (SUNFLOWER; OCT. 3 TO NOV. 1, 1941)

	2.30	1.32	2.11	2.13	1.86	1.94	
.0002	4.06	1.69	2.86	3.35	1.75	2.74	
.0004	2.77	2.62	3.34	2.91	2.62	2.85	
.0006	3.07	4.16	2.80	3.49	2.83	3.27	
.0008	1.99	4.55	2.26	3.21	2.41	2.88	0.016
.0016	3.25	3.45	2.89	4.46	2.72	3.35	
.0060	2.83	3.05	2.45	3.26	2.20	2.76	
.0120	1.85	2.03	2.64	1.75	2.46	2.15	
.0240	.60	.35	.47	.62	.56	.52	

In experiments 3, 4, and 5 with soil colloids, treatments were introduced to show whether the toxicity of boric acid is affected by the following changes in the basic fertilizer: A decrease in the quantity of sulfate, a decrease in the quantity of phosphate, and a substitution of nitrate nitrogen for that part of the nitrogen supplied as ammonium. In experiment 3, with millet, the sulfate was reduced by substituting ammonium chloride for part of the ammonium sulfate. In experiment 4, with wheat, the basic fertilizer was varied only with respect to the quantity of monocalcium phosphate supplied. In experiment 5, with millet, an all-nitrate fertilizer was compared with the standard fertilizer mixture containing one-third of the nitrogen as ammonium. The all-nitrate fertilizer had the composition given for No. 5 in a previous publication.[7] Absorption by the roots generates alkalinity from the all-nitrate fertilizer during the whole growth period, whereas from the standard fertilizer acidity is developed during early growth and alkalinity later, when the ammonium ions become depleted. The results of these experiments are shown in tables 2, 3, and 4.

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[7] GILE, P. L., and FEUSTEL, I. C. EFFECT OF SOIL AND PEAT ADMIXTURES ON THE GROWTH OF PLANTS IN QUARTZ SAND. Jour. Agr. Res. 66: 49–65. 1943. (See p. 50.)

TABLE 2.—*Influence of the sulfate supply on toxicity of boric acid to millet, experiment 3, Oct. 16 to Nov. 18, 1941*

Medium in which plants were grown	Sulfate (SO₄) added per pot	Boron added per pot	Air dry yield of individual pots				Average air-dry yield per pot	Boron per pot required to reduce yield one-half
	Gram	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*
Quartz sand only	0.240	0	4.34	4.88	4.71	5.26	4.80	
		.012	2.23	2.29	2.03	2.24	2.20	0.011
		.024	.27	.71	.54	.51	.51	
	.048	0	4.40	4.52	4.48	4.80	4.55	
		.012	1.68	2.20	1.77	1.76	1.85	.010
		.024	.45	.50	.19	.54	.42	
Sand and Nacogdoches soil	.240	0	5.95	6.89	6.48	6.10	6.36	
		.012	2.74	2.73	3.12	2.30	2.72	.011
		.024	.53	.54	.59	.48	.54	
	.048	0	4.95	6.25	5.87	6.62	5.92	
		.012	2.36	2.65	2.58	2.92	2.63	.011
		.024	.28	.25	.44	.39	.34	

TABLE 3.—*Influence of the phosphate supply on toxicity of boric acid to wheat, experiment 4, Nov. 18 to Dec. 29, 1941*

Medium in which plants were grown	Phosphate (P₂O₅) added per pot	Boron added per pot	Air-dry yield of individual pots					Average air-dry yield per pot	Boron per pot required to reduce yield one-half
	Gram	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*
Quartz sand only	0.12	0.0002	4.04	3.81	4.17	4.30	4.27	4.12	
		.0162	2.45	2.18	2.70	2.84	2.37	2.51	0.021
		.0322	.97	.82	1.00	1.13	1.09	1.00	
	.24	.0002	4.05	4.21	4.08	4.30	4.30	4.17	
		.0162	2.31	2.57	2.65	2.65	2.27	2.49	.021
		.0322	1.30	1.23	.99	1.35	1.35	1.24	
Sand and Muskingum soil	.24	.0002	5.38	4.93	4.86	5.14	4.80	5.02	
		.0162	3.50	3.58	3.42	3.68	3.60	3.56	.029
		.0322	2.32	2.13	2.34	2.20	2.33	2.26	

TABLE 4.—*Influence of the form in which nitrogen is supplied on toxicity of boric acid to millet, experiment 5, Sept. 20 to Oct. 14, 1941*

Medium in which plants were grown	Boron added per pot	Air-dry yield of individual pots				Average air-dry yield per pot	Boron per pot required to reduce yield one-half
	Gram	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*
Quartz sand only, standard fertilizer	0	4.73	4.45	4.54	4.90	4.66	
	.012	2.97	2.56	2.92	2.50	2.74	0.016
	.024	1.62	1.24	1.37	1.24	1.37	
Sand and Conowingo soil, standard fertilizer	0	6.07	4.97	4.86	5.15	5.26	
	.012	2.47	2.84	3.30	2.88	2.87	.014
	.024	.94	.88	.99	.99	.95	
Sand and Conowingo soil, all-nitrate fertilizer	0	7.50	7.47	8.30	7.72	7.75	
	.012	4.48	4.12	4.03	3.89	4.13	.013
	.024	.72	1.00	1.00	1.30	1.01	
Sand and Muskingum soil, standard fertilizer	0	7.20	7.12	7.20	6.62	7.04	
	.012	4.65	4.38	4.17	4.90	4.53	.016
	.024	1.44	1.19	1.59	1.22	1.36	
Sand and Muskingum soil, all-nitrate fertilizer	0	7.86	8.75	8.12	8.10	8.21	
	.012	4.15	4.98	4.57	4.82	4.63	.014
	.024	1.70	1.51	1.91	1.62	1.69	

It appears from tables 2 and 3 that wide variations in the quantities of sulfate or phosphate, above the quantities needed for normal growth, do not affect the toxicity of boric acid, and it seems from table 4 that the toxicity is also unaffected by appreciable changes in the reaction of the absorption zone of the roots. How widely absorption zones of roots exposed to the two fertilizers differed in reaction is not known,[8] but the comparative yields of check pots receiving the two fertilizers show that the difference was sufficient to affect growth appreciably. The Conowingo and Muskingum soils were both acid, having hydrogen-ion concentrations of pH 5.7 and 5.8, respectively.

Four other experiments were conducted, Nos. 6, 7, and 8 with millet and No. 9 with wheat, to determine whether soil colloids affect the toxicity of boric acid. The results are shown in table 5. The standard nutrient salt mixture was applied to all pots except the Colby series in experiment 6, where a salt mixture was used in which all the nitrogen was supplied as ammonium sulfate and potassium chloride was substituted for potassium nitrate; otherwise the mixture was the same as the standard. The Colby soil contains considerable calcium carbonate. This induces a chlorosis in millet but not in wheat, when the standard salt mixture containing two-thirds of the nitrogen as nitrate is used. When the all-ammonium salt mixture is used, probably sufficient acid is developed to maintain availability of the iron.[8]

The effect of soil colloids on the toxicity of boric acid may be judged from the half-toxicity values shown in table 6. Values shown in this table were taken from data given in tables 2, 3, 4, and 5.

It will be seen from table 6 that the half-toxicity values obtained for boric acid in sand-soil mixtures were almost identical with those obtained in pure quartz sand, except for two results with wheat. Evidently, the soil colloids had no appreciable effect on the toxicity of boric acid. If there was any fixation or precipitation of borate ions, it must have been in a form available to the roots or in amounts so small as to be negligible in comparison with the quantity required to reduce growth by one-half. This generalization is based on the results obtained with only eight soil materials, but it should be widely applicable since these soils contained colloidal materials that differed greatly in chemical composition. As shown in column 5 of table 6, the silica-sesquioxide ratios of the colloids ranged from 1.07 to 3.41.

Since the soil colloids do not fix borates in an unavailable form, wide variations in the toxicity of boron in the field would not be expected, at least no such variations as occur in the toxicity of arsenates. Seemingly, in the field, variations in the toxicity of borates would be governed largely by the rainfall and by those properties of the soil that affect the water relations and the movement of salts. Differences in these conditions were largely eliminated in the experiments reported here. The physical properties of the different soil-sand mixtures were not all the same, but they did not vary greatly since the quantity of soil material added to the sand was in all cases small, supplying only 1 percent of colloidal material.

In experiments 4 and 9 (tables 3 and 5), conducted with wheat, the toxicity of boric acid seemed to be slightly reduced by the Muskingum and Colby soil colloids. This, however, was probably due to an error

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[8] See footnote 7, p. 341.

in growing the plants a few days too long, until an essential nutrient was depleted in the check pots. This would enable the plants injured by boron to commence overtaking the checks and thus increase the half-toxicity value. The error would not apply to the quartz-sand series, in which growth was less than in the Colby and Muskingum series.

TABLE 5.—*Toxicity of boric acid to millet and wheat in quartz sand and in sand-soil mixtures*

[All experiments except 9 with millet]

EXPERIMENT 6 (FEB. 9 TO MAR. 11, 1942)

Medium in which plants were grown	Boron added per pot	Air-dry yield of individual pots					Average air-dry yield per pot	Boron per pot required to reduce yield one-half
	Gram	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*
Quartz sand only	0.0002	4.99	5.09	6.36	5.80	--------	5.56	
	.0122	1.27	2.02	1.52	1.65	--------	1.62	0.009
	.0242	.13	.13	.23	.25	--------	.19	
Sand and Colby soil	.0002	7.24	6.92	7.69	7.66	--------	7.38	
	.0122	2.50	1.92	2.92	2.68	--------	2.51	.009
	.0242	.34	.00	.47	.08	--------	.22	
Sand and Manor soil	.0002	6.81	7.57	7.44	6.71	--------	7.13	
	.0122	1.88	2.09	2.40	2.76	--------	2.28	.009
	.0242	.00	.00	.08	.28	--------	.09	
Sand and Kirvin (A horizon) soil	.0002	8.43	7.98	9.40	10.20	--------	9.00	
	.0122	4.53	4.52	4.96	4.50	--------	4.63	.012
	.0242	.42	1.00	.41	.76	--------	.65	

EXPERIMENT 7 (MAR. 23 TO APRIL 16, 1942)

Quartz sand only	0.0002	3.57	3.90	4.10	3.52	2.88	3.59	
	.0122	1.40	1.49	1.52	1.84	1.62	1.57	0.011
	.0242	.10	.11	.26	.23	--------	.18	
Sand and Spearfish soil	.0002	7.36	8.10	7.48	7.50	8.26	7.74	
	.0122	3.75	3.56	3.57	3.15	3.95	3.60	.011
	.0242	.82	.83	.82	.29	--------	.69	

EXPERIMENT 8 (NOV. 18 TO JAN. 4, 1941)

Quartz sand only	0	2.35	1.90	1.87	--------	--------	2.04	
	.012	.67	.93	.95	--------	--------	.85	0.011
	.024	.13	.33	.30	--------	--------	.25	
Sand and Kirvin (B horizon) soil	0	1.28	1.75	1.25	--------	--------	1.42	
	.012	.72	.65	.65	--------	--------	.67	.011
	.024	.21	.20	.14	--------	--------	.18	

EXPERIMENT 9 (DEC. 8 TO JAN. 15, 1942)

Quartz sand only	0.0002	4.08	4.00	4.30	4.13	4.09	4.12	
	.0162	2.32	2.18	2.17	2.42	2.27	2.27	0.020
	.0322	1.48	1.36	1.45	1.35	1.00	1.33	
Sand and Colby soil	.0002	4.95	4.90	4.71	5.00	4.58	4.83	
	.0162	3.31	3.22	3.25	3.45	3.30	3.31	.026
	.0322	1.90	1.85	1.46	1.90	1.73	1.77	
Sand and Nacogdoches soil	.0002	4.61	4.35	4.53	4.06	4.35	4.38	
	.0162	2.15	2.42	2.63	2.30	2.31	2.36	.019
	.0322	1.43	1.58	1.33	1.28	1.30	1.38	

From the preceding experiments it seems that in quartz sand wheat is considerably more resistant than millet to boron toxicity. The half-toxicity values for wheat in experiments 4 and 9 average 0.021 gm. of boron per pot, whereas the average value for millet in six experiments was 0.012 gm. The value for wheat was thus 1.8 times the

value for millet.   A similar relation between wheat and millet holds
for selenate and selenite toxicities.   Previous work[9] shows that wheat
in quartz sand requires 2.3 times the selenate and 1.6 times the sele-
nite that millet requires for half reduction in growth.

TABLE 6.—*Effect of soil colloids on toxicity of boric acid to millet and wheat*

Crop and kind of soil	Boron per pot required to reduce yield in sand-soil mixture by one-half	Boron per pot required to reduce yield in quartz sand by one-half	Difference in boron required, due to soil colloidal material	Molecular ratio of SiO_2 to $Al_2O_3 + Fe_2O_3$ in colloidal material
	Gram	*Gram*	*Gram*	
Millet:				
Colby	0.009	0.009	0	3.41
Conowingo	.014	.016	−.002	2.32
Kirvin, A horizon	.012	.009	+.003	2.02
Kirvin, B horizon	.011	.011	0	1.74
Manor	.009	.009	0	1.81
Muskingum	.016	.016	0	2.26
Nacogdoches	.011	.011	0	1.07
Spearfish	.011	.011	0	2.84
Wheat:				
Colby	.026	.020	+.006	3.41
Muskingum	.029	.021	+.008	2.26
Nacogdoches	.019	.020	−.001	1.07

The data of table 6 indicate that greenhouse conditions exerted
some effect on boron toxicity.   It can be seen that in the experiments
with millet the half-toxicity values for boric acid in pure quartz sand
range from 0.016 gm. of boron per pot to 0.009 gm.   This range in
values obviously is not wholly due to experimental error, since in any
single experiment the half-toxicity values of boric acid in different
mediums are almost the same.   Possibly the duration and intensity
of the sunlight affect the toxicity of boric acid.   Eaton [10], in an exten-
sive series of experiments, observed similar variations in boron toxicity
and explained them similarly.

The quantities of boric acid found producing half-injury in these
experiments may have no general significance beyond the object of
this particular study.   For this reason, the toxicity values have been
expressed as grams of boron per 1-gallon pot rather than as parts per
million of sand or water.   The average quantity of boron per pot pro-
ducing half-injury to millet in the quartz-sand cultures, 0.012, was
equivalent to about 2.4 parts of boron per million of sand, 16 parts of
boron per million of water, or 130 pounds of borax per acre.

SUMMARY

Pot experiments were conducted to determine the effect of soil col-
loids on the toxicity of boric acid.   Foxtail millet and wheat were
grown in pure quartz sand and in sand-soil mixtures with applications
of boric acid.

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[9] See footnotes 3 and 4, p. 339.
[10] EATON, F. M. DEFICIENCY, TOXICITY, AND ACCUMULATION OF BORON IN PLANTS. Jour. Agr. Res. 69: 237-
277, illus. 1944.

The sand-soil mixtures contained sufficient soil to supply 1 percent of soil colloids. Eight different soil materials were used; these contained colloidal materials with silica-sesquioxide ratios ranging from 1.07 to 3.41. The toxicity of boric acid in each soil-sand mixture was almost exactly the same as in pure quartz sand. It thus appears that soil colloids do not fix boric acid in a form unavailable to the plants.

The toxicity of boric acid was also unaffected by variations in the quantities of sulfate and phosphate in the basic fertilizer and by substitution of an all-nitrate fertilizer for one containing two-thirds of the nitrogen as nitrate and one-third as ammonium.

TURNIP MOSAIC VIRUSES [1]

By Francis J. LeBeau, formerly *research assistant,* and J. C. Walker, *professor, Department of Plant Pathology, Wisconsin Agricultural Experiment Station* [2]

INTRODUCTION

The first transmissible virus diseases demonstrated on cruciferous plants were found in nature on turnip (*Brassica rapa* L.). They were reported in Indiana in 1921 by Gardner and Kendrick (5) [3] and from Washington, D. C., by Schultz (14). In the subsequent 20 years turnip mosaic was recorded from New York (4), Wisconsin (8), and Florida (23) in the United States, and from the Philippine Islands (1), Australia (13), New Zealand (2, 3), Japan, (7, 15), China (12), South Rhodesia (9), and Denmark (6). Since the work of Hoggan and Johnson (8), published in 1935, more attention has been given to the differentiation and characterization of viruses on cruciferous plants. As a result a number of distinct viruses or strains of viruses have been described on horseradish (*Armoracia rusticana* Gaertn., Mey., and Scherb.) (8), cauliflower (*Brassica oleracea* L. var. *botrytis* L.) (16), cabbage (*B. oleracea* L. var. *capitata* L.) (10, 11, 20, 22), Chinese cabbage (*B. pekinensis* (Lour.) Rupr.) (21), radish (*Raphanus sativus* L.) (19), annual stock (*Matthiola incana* (L.) R. Br. var. *annua* (L.) Voss (18), rape (*B. napus* L.) (12) and turnip (8, 17).

It is a significant fact that turnip was found to be host for each of these as they were described. In all cases distinct mottle or vein clearing or both occurred, and the plants were rather severely affected. On the other hand, each virus was distinguishable from the others on the basis of differences either in properties or host range or both.

In the course of studies on cabbage mosaic at Madison, Wis., reported recently (22), turnips showing signs of virus infection were commonly observed. Isolates from this host were made for comparison with those of cabbage mosaic. Isolates were also secured from the experimental plots at the Louisiana Agricultural Experiment Station. Of several collections four were studied critically. One of these conformed closely with cabbage virus A described recently (22), but the remaining three were distinct in certain respects from crucifer viruses previously described. The present paper reports details of the study of the four virus strains isolated from turnip.

METHODS AND MATERIALS

The methods of study of the viruses were similar to those recently detailed in another paper (22). The virus strains used in this investigation were as follows:

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(347)

Strain T1 was isolated from naturally infected turnip at the Louisiana Agricultural Experiment Station in March, 1939. At that time an isolation from cultivated mustard (probably *Brassica nigra* (L.) Koch) was also made but when found to be apparently similar to T1 it was discarded.

Strains T6, T8, and T9 were isolated from naturally infected turnip in experimental plots adjoining cabbage mosaic plots at the Wisconsin Agricultural Experiment Station in 1938 and 1939.

Stock cultures of isolates T6, T8, and T9 were kept on plants of *Nicotiana glutinosa* L. and cultures of T1 on turnip plants in insect-proof cages. Seedlings were usually inoculated in the third- or fourth-leaf stage and incubated at temperatures ranging from 24° to 28° C. Uninoculated controls were always carried for comparison with inoculated plants. Reisolations were attempted from all plants tested as possible hosts by inoculation to turnip or tobacco. The cabbage aphid (*Brevicoryne brassicae* (L.)) and the green peach aphid (*Myzus persicae* (Sulz.)) were used as insect vectors.

EXPERIMENTAL RESULTS

SYMPTOMS OF THE DISEASES

IN THE FIELD

Observations were made at Madison, Wis., in naturally infected plots from which T6, T8, and T9 were isolated. Since the symptoms later produced by these strains on turnip in the greenhouse were quite similar, it is assumed that this description of field symptoms will apply to the disease as produced by any one of the strains. After midsummer the disease was very severe. The affected areas in turnip plots were usually more or less circular in shape with severely stunted or dead plants at the center, indicating that infection had spread progressively outward from an initial center. Newly unfolding leaves of severely infected plants were dwarfed, malformed, and mottled while some leaves became entirely chlorotic, except for occasional, irregularly-shaped, raised islands of normal green or green darker than normal. Necrosis varied from small, more or less circular spots, to large, irregular areas and streaks, which finally involved whole leaves, causing them to dry up and absciss prematurely. When progressive dying of the older leaves continued, only a rosette of dwarfed, malformed leaves remained. Such leaves were not always severely mottled but were frequently darker green than normal throughout. The enlargement of the root and hypocotyl under these conditions was greatly suppressed. On the older leaves of less severely infected plants, vein clearing was sometimes evident, supplanted later by fine interveinal mottling. When White Milan and Purple Top White Globe varieties were grown side by side in the field at Madison, the former was much more severely affected (fig. 1).

When the mosaic disease was observed in December at Baton Rouge, La., in plots from which T1 was isolated, the symptoms were relatively mild as compared with those at Madison in midsummer. This may have been due in part to the cooler weather at Baton Rouge since it will later be shown that these mosaic isolates produced a severer type of disease above 20° C. than below. It was also undoubtedly due

FIGURE 1.—White Milan (left) and Purple Top White Globe (right) varieties of turnip naturally infected in plots at Madison, Wis., from which strains T6, T8, and T9 were isolated. Note that the greater susceptibility of White Milan to damage from mosaic has resulted in the death of most plants while the plants of Purple Top White Globe, though generally affected, are not seriously stunted

in part to the lack of virulence of T1 on turnip. Although a high percentage of the plants in the plot were infected, it was necessary to examine individual plants to detect the disease. Coarse mottled or irregular, frequently puckered, normal green or darker than normal green areas on a slightly chlorotic background occurred, but neither severe malformation nor necrosis was apparent. Little stunting could be detected.

IN THE GREENHOUSE

Young plants of the varieties White Milan and Purple Top White Globe were inoculated in the greenhouse with each isolate and kept at a constant temperature of 24° C. It was evident that White Milan and Purple Top White Globe were about equally affected by T1, but that White Milan was, as in the field, much more severely affected by T6, T8, and T9. T1 was much less severe on both varieties than T6, T8, and T9, as is shown for Purple Top White Globe in figure 2.

On plants inoculated with T1, vein clearing persisted on the first three or four leaves to develop symptoms, but as new leaves unfolded, it was soon replaced by a coarse mottle or by green bands along the veins (fig. 3). Necrotic spots were rare, but older leaves turned yellow and dried up prematurely. Aerial parts were only slightly dwarfed, but the root and hypocotyl were stunted considerably. The disease was never lethal.

The symptoms produced by T6, T8, and T9 were very much alike. Necrotic lesions on the inoculated leaf were noted only with isolate T9. These appeared in 5 to 8 days as small, circular, reddish-brown lesions, which later enlarged to a diameter of 2 to 3 mm. Only a

small percentage of inoculated plants gave rise to such symptoms, and in a number of inoculation series no lesions were observed. Usually they occurred only when old leaves were inoculated and then only at 24° and 28° C. Systemic invasion by either isolate was first apparent as a fine vein clearing, accompanied by a marked
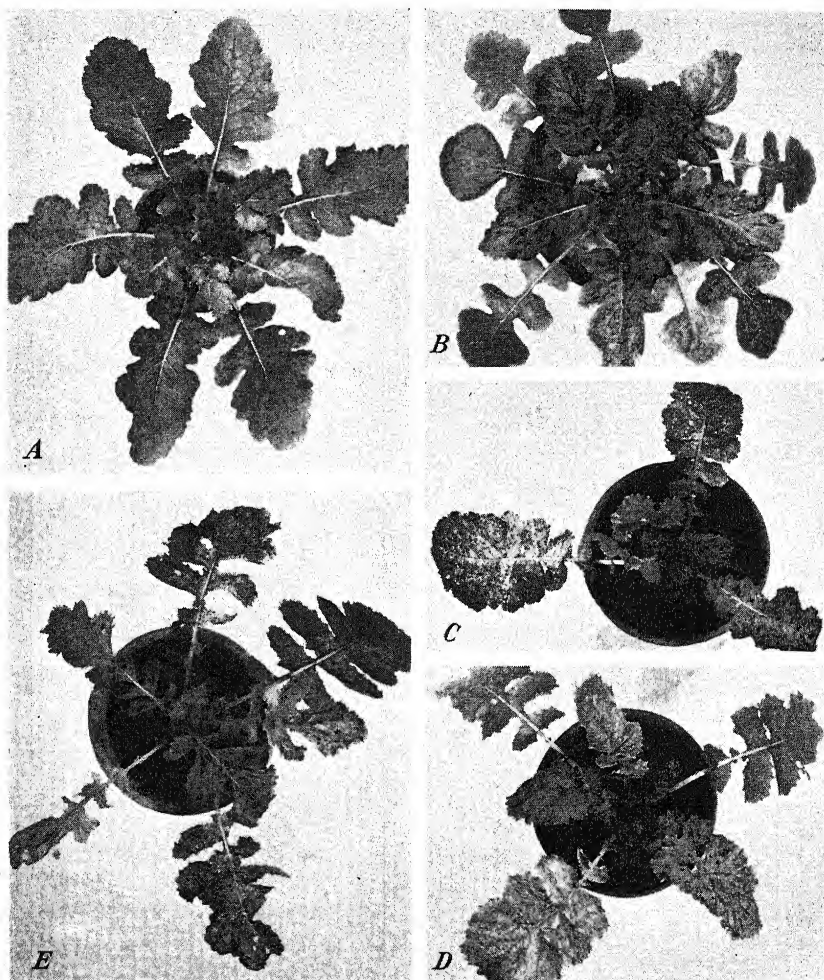
FIGURE 2.—Young turnip plants of the Purple Top White Globe variety inoculated with isolates T1, T6, T8, and T9 and kept in the greenhouse for 20 days at 24° C. *A*, uninoculated control. *B*, Plant inoculated with T1; note coarse mottle on younger leaves with little suppression of growth. *C, D, E*, Plants inoculated with T6, T8, and T9, respectively; note severe stunting, chlorosis, and leaf malformation with each of these isolates.

crinkling of the youngest leaves. Vein clearing did not persist but was rapidly replaced by a fine interveinal mottle in the case of T6 and T9. With T8, as newer leaves unfolded, they were malformed and severely mottled, becoming chlorotic throughout as they ex-
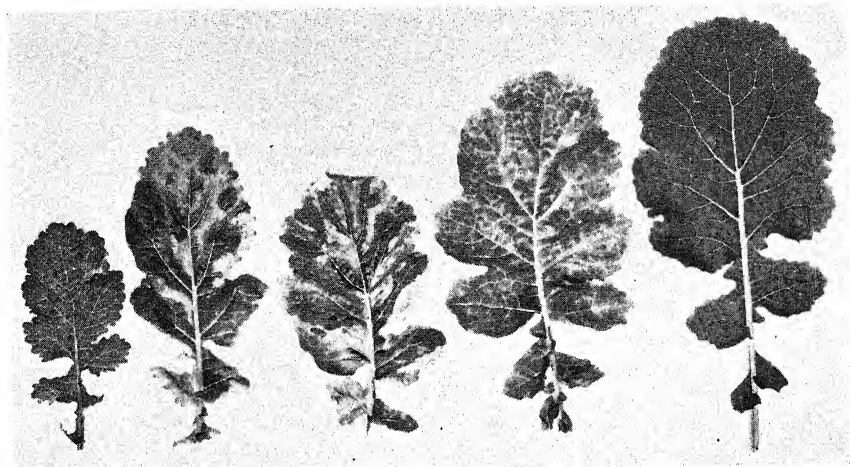
FIGURE 3.—Symptoms on Purple Top White Globe turnip inoculated with isolate T1 and grown at 24° C. for 35 days; leaf from uninoculated plant at right; successively older leaves from inoculated plant, from left to right. Note chlorosis and slight vein clearing in oldest and youngest leaf, with chlorosis and prominent vein banding in the other two leaves.
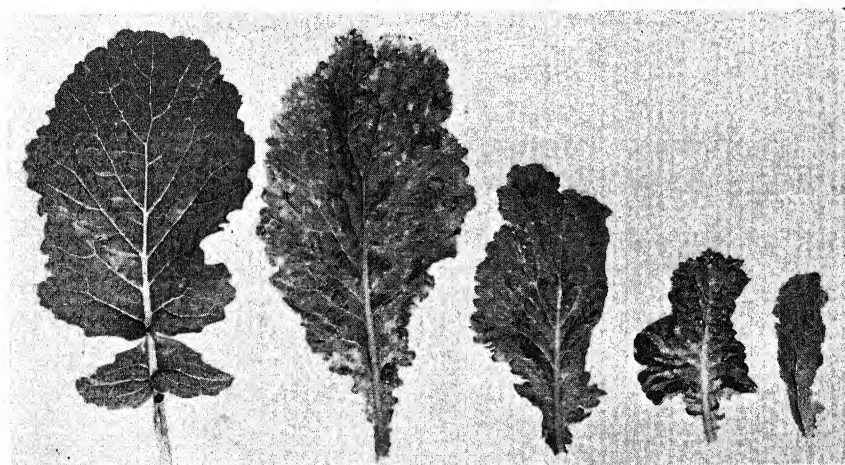
FIGURE 4.—Symptoms on White Milan turnip inoculated with isolate T8 and kept 22 days at 24° C.; leaf from uninoculated plant at left, and successively younger leaves from inoculated plant, from left to right. Note crinkling, malformation, and severe mottling of youngest leaves, with decided chlorosis of older ones.

panded. Stunting was less severe with T9 than with T6 or T8, and the leaves tended to become filiform as they expanded (fig. 4). Necrosis resulting from systemic invasion usually set in on the older leaves in local areas, which rapidly enlarged and coalesced to involve the whole leaf. It was considerably more severe in White Milan than in Purple Top White Globe. T6, T8, and T9 killed many of the former; none of the isolates was lethal to Purple Top White Globe.

RELATION OF TEMPERATURE TO EXPRESSION OF SYMPTOMS

Symptom expression in White Milan and Purple Top White Globe, when infected with the four virus isolates at constant greenhouse temperatures of 16°, 20°, 24°, and 28° C., was studied in three series of tests. Each of the two turnip varieties was most severely affected by each isolate at the highest of these temperatures. At 28° the symptoms did not differ greatly from those at 24°, but they usually appeared earlier and the course of disease development was more rapid. Isolates T6, T8, and T9 were often lethal to the variety White Milan at 24° and 28° but not to Purple Top White Globe. T1 never caused death of plants of either variety. At 16° and 20° there was partial masking of the symptoms produced on each variety by each isolate. At 20° vein clearing, mild mottling, and slight dwarfing occurred in plants infected with T1, T6, and T8; T9 was more severe. At 16° T1, T6, and T8 produced only vein clearing, and mottling appeared but rarely; with T9 vein clearing, mild mottling, and slight stunting were of common occurrence.

PHYSICAL PROPERTIES

The thermal inactivation point, tolerance to dilution, and longevity in vitro were determined for each isolate. The data secured are given in table 1. Since T6, T8, and T9 caused local lesions on tobacco, the local-lesion method was used in all cases with those three viruses and the turnip inoculation test was applied in addition in the thermal inactivation determination. The two methods produced very similar results. T1 was tested only on turnip since tobacco was not infected by this isolate.

TABLE 1—*Properties of 4 turnip viruses as determined by the number of local lesions formed on inoculation to tobacco or the number of systemically infected plants on inoculation to turnip*

Type and degree of treatment	Number of local lesions on tobacco inoculated with virus [1]—			Number of turnip plants infected out of 16 plants inoculated with virus [2]—			
	T6	T8	T9	T6	T8	T9	T1
Undiluted	2,289	1,914	2,004	---------	---------	---------	15
Dilution:							
1–10	303	214	229	---------	---------	---------	15
1–100	82	51	66	---------	---------	---------	15
1–1,000	26	5	18	---------	---------	---------	10
1–2,000	-------	1	---------				
1–5,000	13	0	3	---------	---------	---------	6
1–10,000	1	0	1	---------	---------	---------	3
1–50,000	0	0	0	---------	---------	---------	0
Untreated	3,994	3,706	4,978	14	12	14	14
Heating for 10 minutes (°C.):							
50°	613	265	516	11	7	9	10
52°	256	99	153	9	6	7	11
54°	40	31	41	5	3	4	5
56°	0	1	1	0	1	1	0
58°	0	0	0	0	0	0	0
Inoculated immediately	3,541	2,524	2,134	---------	---------	---------	15
Aging in vitro at 20° C.:							
1 day	1,659	2,323	1,406	---------	---------	---------	-------
2 days	382	394	212	---------	---------	---------	-------
3 days	79	264	37	---------	---------	---------	-------
4 days	33	120	3	---------	---------	---------	10
5 days	6	68	1	---------	---------	---------	8
6 days	4	26	0	---------	---------	---------	6
7 days	0	3	0	---------	---------	---------	6
8 days	0	0	0	---------	---------	---------	3
9 days							1
10 days							0
11 days							0

[1] Average of 3 trials.   [2] Average of 2 or 3 trials.

The four isolates were quite similar in reaction to these tests. T8, which will be shown later to conform most closely to cabbage mosaic virus A (22), was slightly less tolerant to dilution than T1, T6, or T9. T1 and T6 were inactivated at 56°; T8 and T9 at 58°; T8 was inactivated at 96 hours in vitro at 20°, T6 at 84 hours, T9 at 72 hours, and T1 at 120 hours. It is doubtful that any of these differences are significant, and they could not be depended upon to distinguish between the isolates.

INSECT TRANSMISSION

Each virus isolate was found to be transmissible by the green peach aphid and the cabbage aphid. A high percentage of turnip plants inoculated with either virus isolate by either of the insect species in several trials became infected and produced typical symptoms of the respective virus isolates. Each virus isolate was reisolated by mechanical means from plants infected by aphids. Control plants exposed to nonviruliferous insects remained healthy

HOST RANGE

In table 2 are summarized the results of inoculation of each isolate to a number of cruciferous and noncruciferous plants upon which the reaction of other viruses from cruciferous hosts has been recorded. Since the study on turnip indicated that these were relatively high temperature viruses, these tests were made at 24° and 28° C. It may be seen that T8 infected all botanical varieties of *Brassica oleracea* tested while the other three forms were much more restricted. T1 and T6 infected Italian Green Sprouting broccoli, but did not infect kohlrabi, collard, cabbage, cauliflower, kale, or brussels sprouts. T9 infected kohlrabi and collard as well as Italian Green Sprouting broccoli but did not infect cabbage, cauliflower, kale, or brussels sprouts. All four isolates infected rutabaga, white mustard, black mustard, leaf mustard, rape, and Chinese cabbage, and there was little distinction between the four in symptoms on these hosts except that T1 tended often to produce milder symptoms than any of the other three. Annual stock was infected only by T8 and dames violet only by T8 and T9.

TABLE 2.—*Symptoms produced on young plants in the greenhouse when inoculated mechanically with virus isolates T1, T6, T8, and T9 from turnip*

Host	Symptoms resulting from inoculation with isolate—			
	T1	T6	T8	T9
Cruciferae:				
Brassica oleracea L. var. *capitata* L. (cabbage, var. Jersey Queen).	None.	None.	Local: None. Systemic: Early, slight vein clearing; small, light-green, chlorotic spots, changing to a coarse, irregular mottle; occasional necrosis.	None.
B. oleracea var. *botrytis* L. (cauliflower, var. Early Snowball).	None.	None.	Local: None. Systemic: Same as for cabbage, except no necrosis.	None.
B. oleracea var. *botrytis* L. (broccoli, var. Italian Green Sprouting).	Local: None. Systemic: Very mild chlorotic spots, visible only by transmitted light.	Same as for T8.	Local: None. Systemic: Same as for cabbage, except no necrosis.	Same as for T8.
B. oleracea var. *gongylodes* L. (kohlrabi, var. Early White Vienna).	None.	None.	Local: None. Systemic: Same as for cabbage, except no necrosis.	Local: None. Systemic: Faint, circular, chlorotic spots, visible only by transmitted light; becoming less discernible with age.
B. oleracea var. *viridis* L. (kale, var. Dwarf Scotch Curled).	None.	None.	Local: None. Systemic: Same as for cabbage, except chlorotic spots remain as distinct, light-green, circular areas.	None.
B. oleracea var. *viridis* L. (collard, var. Southern Georgia or Creole).	None.	None.	Local: None. Systemic: Same as for cabbage.	Local: None. Systemic: Same as for kohlrabi.
B. oleracea var. *gemmifera* DC. (Brussels sprouts, var. New Danish).	None.	None.	Local: None. Systemic: Same as cabbage, with necrosis more severe as small, circular, reddish-brown spots on older leaves.	None.

Host				
B. campestris L. var. *napobrassica* (L.) DC. (rutabaga, var. American Purple Top).	Local: None. Systemic: Faint, diffuse, chlorotic spots on young leaves, developing into diffuse mottle of faint, chlorotic, and light-green areas.	Same as for T8.	Local: Erratic occurrence of local lesions. Systemic: Some plants become flaccid and leaves necrotic with no mottle; others show pronounced mottle; dwarfing pronounced.	Same as for T8.
B. campestris L. (yellow mustard).	Not tested.	Local: None. Systemic: Same as for T8, except less severe stunting.	Local: None. Systemic: Fine vein clearing on first 3 or 4 leaves; subsequent young leaves show only coarse mottle; severe stunting.	Local: None. Systemic: Same as for T8.
B. hirta Moench (*B. alba* (L.) Rabenh.) white mustard).	Local: None. Systemic: Same as for T8 with less severe stunting.	Local: None. Systemic: Same as for T8 with less severe stunting.	Local: None. Systemic: Same as for yellow mustard.	Local: None. Systemic: Same as for T8.
B. nigra (L.) Koch (black mustard).	Local: None. Systemic: Vein-clearing on successive new leaves; well-defined mottle rare.	Local: None. Systemic: Same as for T8.	Local: None. Systemic: Vein clearing, changing rapidly into severe, fine, yellow, interveinal mottle; progressive yellowing and dying of older to younger leaves, with no other symptoms, or mosaic mottle followed by death or severe stunting.	Local: None. Systemic: same as for T1.
B. juncea (L.) Coss. (leaf mustard, var. Tender Green).	Local: None. Systemic: Vein clearing, continuing on successive leaves; leaf malformation and dwarfing less pronounced than with T8.	Local: None. Systemic: Same as for T8.---	Local: None. Systemic: Vein clearing on first 2 or 3 leaves, followed by fine interveinal mottle; subsequent leaves much dwarfed, curled, malformed, chlorotic with dark-green islands.	Local: None. Systemic: Same as for T8.
B. napus L. (rape, var. Dwarf Essex).	Local: None. Systemic: Vein clearing, superseded by very mild chlorosis (associated with absence of bloom) with scattered normal green areas; successive leaves developing coarser mottle.	Local: None. Systemic: Same as for T1.	Local: None. Systemic: Vein clearing, rapidly superseded by interveinal mottle and finally a chlorotic background with scattered, small, green areas, more severe at base and in center of leaf blade; dwarfing.	Local: None. Systemic: Same as for T1.
B. pekinensis (Lour.) Rupr. (Chinese cabbage, var. Chihli).	Local: None. Systemic: No dwarfing or malformation; conspicuous mottle of yellow areas in normal green background or dark-green islands, often puckered, in chlorotic background.	Local: None. Systemic: Infection rare, similar to T8.	Local: None. Systemic: Inconspicuous clearing of veins, rapidly replaced by mottle of diffuse chlorotic spots; subsequent leaves crinkled, savoyed, dwarfed, but not conspicuously mottled; necrosis as small spots or streaks along midrib.	Local: None. Systemic: Same as for T8.

TABLE 2.—*Symptoms produced on young plants in the greenhouse when inoculated mechanically with virus isolates T1, T6, T8, and T9 from turnips*—Continued

Host	Symptoms resulting from inoculation with isolate—			
	T1	T6	T8	T9
Cruciferae—Continued. Hesperis matronalis L. (dames violet).	None.	None.	Local: None. Systemic: Vein clearing in first or second leaf; subsequent leaves chlorotic with dark-green islands or bands along veins; marginal inroll of young leaves; dwarfing.	Local: None. Systemic: Same as for T8.
Matthiola incana (L.) R. Br. var. annua (L.) Voss (annual stock, var. Dwarf Ten Weeks).	None.	None.	Local: None. Systemic: Mild interveinal mottle, followed by irregular, necrotic spots and streaks; severe stunting; no flower breaking observed.	None.
Arabis albida Stev. (rockcress).	Not tested.	None.	None.	None.
Cheiranthus allionii Hort. (wallflower).	None.	None.	None.	None.
Non-Cruciferae: Nicotiana tabacum L. (tobacco, var. Connecticut Havana 38).	None.	Local: Same as for T8. Systemic: None.	Local: Lesions in 3 to 4 days on inoculated leaves, enlarging rapidly to 3 mm. or more in diameter, usually showing brick-red center with concentric rings and darker band at edge. Systemic: None.	Local: Same as for T8. Systemic: None.
N. glutinosa L.	Local: None. Systemic: Very mild or none; halo chlorotic spotting, followed by occasional necrosis in old leaves; no perceptible dwarfing.	Local: None. Systemic: Same as for T8.	Local: None. Systemic: Severe chlorotic halo spotting, rapidly becoming necrotic.	Local: Circular yellow spots, 2 to 3 mm., not becoming necrotic; irregular in occurrence. Systemic: Same as for T8.
N. tabacum × N. glutinosa F1,	None.	Same as for N. tabacum.	Same as for N. tabacum.	Same as for N. tabacum.
N. rustica L.	None.	None.	Local: None. Systemic: Small, distinct, circular, faintly chlorotic spots, enlarging as leaf expands; little or no malformation or stunting.	Local: None. Systemic: Same as for T8.

Host plant				
N. repanda Lehm.	None.	Local: Same as for T8. Systemic: Same as for T8, except that in later stage necrotic pattern remains that of individual, circular areas.	Local: Small, light brown spots in 3 to 5 days, enlarging to 5 to 8 mm., extending to involve whole leaf. Systemic: Few small, circular, distinct, chlorotic spots, rapidly becoming necrotic; extending to produce irregular, necrotic pattern and streaks along veins.	Local: Same as for T8. Systemic: Same as for T8.
N. sylvestris Speg.	None.	Same as for *N. tabacum*.	Same as for *N. tabacum*.	Same as for *N. tabacum*.
N. multivalvis Lindl.	Not tested.	Local: None. Systemic: Vein-clearing followed by chlorotic spotting.	Local: None. Systemic: Severe etching or necrosis of veins and veinlets.	Local: None. Systemic: Same as for T6.
Petunia hybrida Vilm. (petunia, var. Violet King).	Local: None. Systemic: Indefinite chlorotic banding of larger veins, followed by bright yellow, well-defined spots, few on a leaf; occasional chlorotic bands along large veins; slight dwarfing; no mottle of flower petal.	Local: None. Systemic: Similar to but less severe than T8; flower symptoms not studied.	Local: None. Systemic: Vein clearing, changing into fine, interveinal mottling; dwarfing; conspicuous mottle of flower petals.	Local: None. Systemic: Similar to but less severe than T8; conspicuous mottle of flower petals.
Beta vulgaris L. var. *cicla* L. (Swiss chard, var. Lucullus).	Local: None. Systemic: Same as for T8.	Local: None. Systemic: Same as for T8.	Local: None. Systemic: Erratic necrotic lesions.	Local: None. Systemic: Same as for T8.
B. vulgaris L. (sugar beet, var. Kleinwanzleben).	Same as for Swiss chard.	Same as for Swiss chard.	Same as for Swiss chard.	Same as for Swiss chard.
Spinacia oleracea L. (spinach, var. Bloomsdale).	None.	Local: None. Systemic: Same as for T8.	Local: None. Systemic: Vein clearing, changing to fine, severe, interveinal mottle with severe malformation and dwarfing of leaves.	Local: None. Systemic: Same as T8.
Zinnia elegans Jacq. (Lilliput zinnia, var. Giant Golden).	Local: None. Systemic: Vein clearing, disappearing with age, replaced by chlorotic bands along large veins and by few faint chlorotic spots near base of leaf blade; no stunting.	Local: None. Systemic: Same as for T8.	Local: None. Systemic: Vein clearing, severe, coarse mottle; stunting.	Local: None. Systemic: Same as for T8.

Among noncruciferous plants quite as wide a divergence occurred. T1 did not affect tobacco while T6, T8, and T9 caused only local lesions. All affected *Nicotiana glutinosa* (fig. 5). T9 was the only one
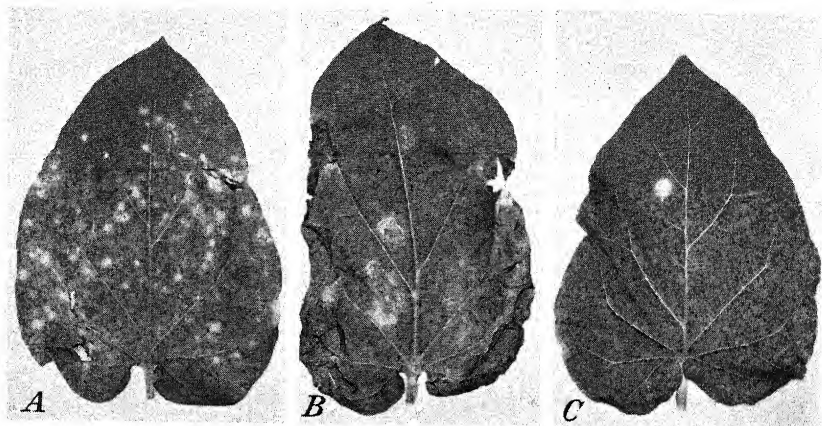
FIGURE 5.—Effect of turnip virus isolates on *Nicotiana glutinosa*. *A*, Circular yellow spots produced by T9 on inoculated leaves after 10 days at 20° C; T1, T6 and T8 did not produce local lesions. *B*, Systemic infection after 20 days at 24° characteristic of T6, T8, and T9; severe chlorotic halos rapidly became necrotic. *C*, Systemic infection by T1 after 22 days at 24°; relatively uncommon chlorotic spots which rarely became necrotic.

to produce local lesions in the form of small yellow spots while systemically it produced symptoms similar to those caused by T8. T6 and T8 caused severe chlorotic halo spots which rapidly became necrotic while T1 uncommonly produced chlorotic spots which rarely
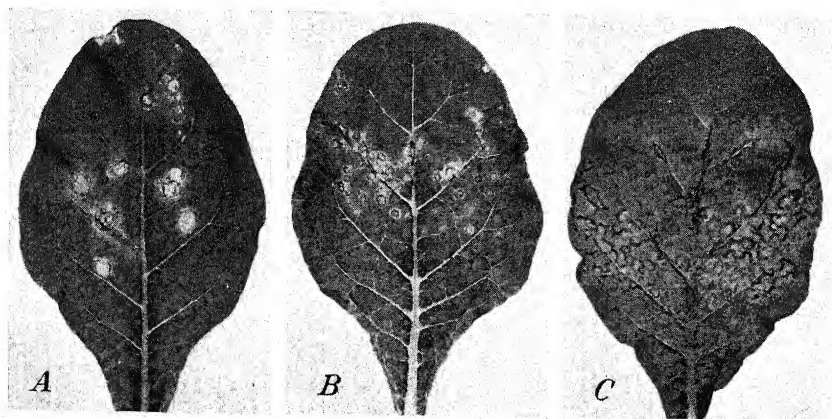
FIGURE 6.—Symptoms on *Nicotiana repanda*. *A*, Local lesions produced on inoculated leaves; these are typical of T6, T8, and T9. *B*, Systemic symptoms of T6; necrotic lesions remain distinct. *C*, Systemic symptoms characteristic of T8 and T9; necrosis of local spots extends to form an irregular pattern and necrosis along veins. T1 causes no infection on *N. repanda*.

became necrotic.   T1 and T6 did not affect *N. rustica* and T1 did not affect *N. repanda* or *N. sylvestris*.   T6, T8, and T9 produced on *N. repanda* local necrotic lesions which enlarged to 5 to 8 mm. in diameter (fig. 6, *A*).   Systemic symptoms appeared as small chlorotic spots which rapidly became necrotic.   With T6 these spots remained distinct (fig. 6, *B*), but with T8 and T9 the necrosis extended to produce irregular patterns and streaks along the veins (fig. 6, *C*).   A striking difference between isolates was shown in the systemic symptoms produced on *N. multivalvis*.   T6 and T9 were similar in that they produced vein clearing followed by chlorotic spotting (fig. 7, *B*); in this
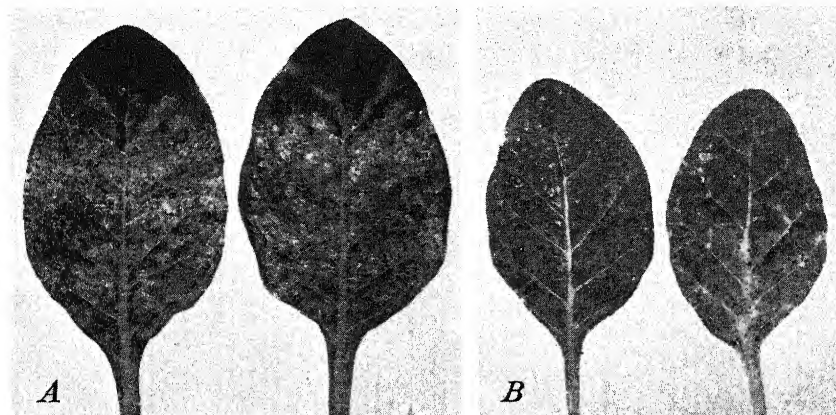
FIGURE 7.—Systemic symptoms on *Nicotiana multivalvis*. *A*, Conspicuous etching pattern caused by isolate T8. *B*, Chlorotic spotting and vein clearing characteristic of T6 and T9.

respect they were similar to cabbage virus A (*22*).   T8, which in most hosts produced symptoms similar to cabbage virus A, caused etching and vein necrosis which assumed a striking pattern (fig. 7, *A*) very much like that produced by the cabbage black ring virus on this host (*22*).

All isolates reacted similarly on beet and chard.   All affected spinach except T1.   All affected petunia and zinnia, but the symptoms produced by T1 differed from those produced by the other three.   In zinnia, T1 symptoms consisted of mild, localized chlorosis, while those of T6, T8, and T9 consisted of severe coarse mottle with considerable stunting of affected leaves (fig. 8).
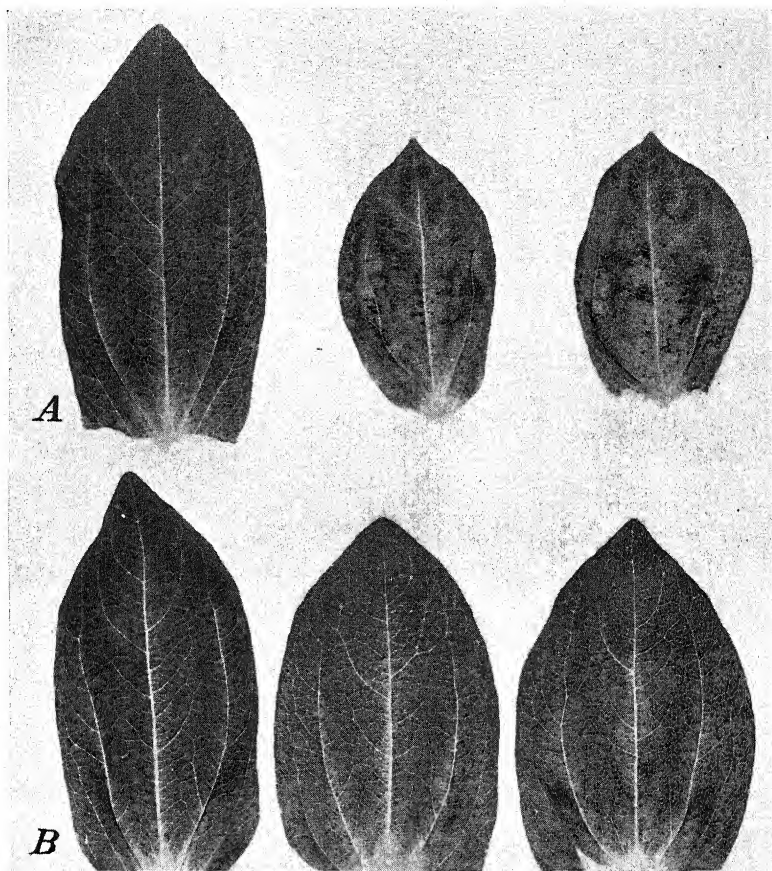
FIGURE 8.—Systemic symptoms on zinnia. *A*, Severe chlorotic mottle characteristic of isolates T6, T8, and T9; leaf from uninoculated plant on left. *B*, Mild localized chlorosis caused by T1; leaf from uninoculated plant on left. Leaves photographed 19 days after inoculation at 24° C.

DISCUSSION

In table 3 are brought together the property reactions and host ranges of the four isolates described in this paper in comparison with others recorded in the literature.   When the symptoms produced by T8 are compared with those recently recorded (*22*) for viruses on cabbage, it is evident that T8 is very similar to cabbage virus A. The latter, together with the black ring virus of cabbage, has been classed as a strain of the turnip virus 1 of Hoggan and Johnson (*22*). T8 is undoubtedly another strain of this group.   In most respects it produced symptoms similar to cabbage virus A, but on *Nicotiana multivalvis* it produced symptoms almost identical with those produced by the black ring virus (fig. 7).

Insofar as T8, cabbage virus A, the ring necrosis virus, and the black ring virus have been compared, they have been found to have nearly identical host ranges.   They differ chiefly in their effects upon

TABLE 3.—*Comparison of properties and host range of isolates T1, T6, T8, and T9 with those of other viruses described from crucifers* [1]

Properties or host range	Turnip virus 1, H and J (8)	Turnip mosaic Tompkins (17)	Turnip mosaic Chamberlain (9)	T1	T6	T8	T9	Cabbage virus A (22)	Cabbage black ring (22)	Cabbage ring necrosis (11)	Radish mosaic (19)	Mild stock mosaic (18)	Severe stock mosaic (18)	Rape mosaic (12)	Cabbage virus B (22)	Cauliflower mosaic (16, 22)	Chinese cabbage mosaic (21)
Properties:																	
Inactivation by dilution	100,000	4,000	1,000	50,000	50,000	5,000	50,000	4,000–10,000	1,000	600	15,000	5,000	4,000	7,000	500–1,500	3,000	6,000
Inactivation by ageing (hrs.)	72	72	72	120	84	96	72		48	48	384	144	192	144		360	96
Inactivation by heat (°C.)	54	63	55–60	56	56	58	58	59–60	59	50	68	60	60	65	74–76	75	75
Host range:																	
Turnip	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rutabaga		+		+	+	+	+	+	+	+	0	+	+		+	+	+
Cabbage	+	+	+	0	+	+	+	+	+	+	+	+	+	0	+	+	+
Cauliflower		+		0	0	+	0	+	+	+	+	+	+	0	+	+	
Green sprouting broccoli		0		+	0	+	0	+	+	+	+	+	+	0	+	+	
Kohlrabi		0		0	0	+	+	+	+	+	+	+	+		+	+	
Kale		0	+	0	0	+	0	+	+	+	+	+	+	+	+	+	+
Brussels sprouts		0		0	0	+	0	+	+	+	+	+	+		+	+	
Rape		0		+	+	+	+	+	+	+	0	+	+	0	+	+	+
White mustard	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
Black mustard		0		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leaf mustard		+		+	+	+	+	+	+	+	+	+	+	0	+	+	
Radish	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chinese cabbage		+				+		+	+	+	+	+	+			+	+
Dames violet		+	+	+	+	+	+	+	+	+	L	L	+	+	+	0	+
Annual stock		+		0	0	L	0	L	L	L	0	L	0	0	0	+	L
Tobacco	L	L		0	0	L	L	+	+	+	L	L	+	+	0	0	L
Nicotiana glutinosa	L?	L		0	0	+	+	+	+	+	L	0	0	0	0	0	
Nicotiana rustica		0		+	+	+	+	+	+	+	+		+		0	0	
Nicotiana repanda				0	+	+	+	+	+	+	+		0		0	0	
Nicotiana sylvestris				+	+	+	+	+	+	+	+		0		0	0	
Nicotiana multivalvis				+	+	+	+		+	+	+		+			0	
Petunia				0	+	+	+			+	+						
Chard										+						0	
Beet		0										0	+	+		0	0
Spinach		0														0	
Zinnia																	0

[1] 0 = No symptoms; + = positive systemic symptoms; L = local lesions on inoculated leaves.

the host plants. T1, T6, and T9 closely resemble the above-named in properties, as distinct from the cauliflower mosaic group, but differ from them and from each other in host range and in symptomatology on certain common hosts. In view of information now at hand on other virus groups, such as the cucumber virus group (*24*), it is not surprising to find closely similar crucifer viruses with rather distinct selective host ranges. It is much more logical to consider T1, T6, T8, and T9 as strains of turnip virus 1 than to describe them as distinct viruses.

As stated in another paper (*22*), cabbage virus B, cauliflower mosaic virus, and Chinese cabbage virus, all of which affect turnip, are closely related and are distinct from the turnip virus 1 group. They are more closely restricted to the Cruciferae, they produce more pronounced vein clearing, they have a lower temperature optimum, and a thermal inactivation point some 15° to 20° higher than those of the turnip virus 1 group.

The question may logically be raised as to how certain one may be that isolates T1, T6, T8, and T9 are single-virus strains or what assurance one may have that their apparent differences are not in fact the result of mixtures of one or more strains. It is probable that with the methods employed in this study such a question cannot be answered finally. It should be pointed out, however, that the possibility of mixture with a virus of the cauliflower-mosaic group, which is the usual mixture when one collects a virus extract from cabbage affected with mosaic in the Middle West, has been removed by the thermal inactivation study (table 1). If such a virus were present, there would have been infection after heating much beyond 60° when turnip was used as a test plant. Furthermore, viruses T6, T8, and T9 were each reisolated from local lesions on tobacco and inoculated to turnip with resulting symptoms typical of the original isolate. Stock cultures were then maintained on *Nicotiana glutinosa* to preclude contamination by strains of the cauliflower-mosaic group.

When turnips are found affected with mosaic in nature, any one of the strains so far fully described from crucifers may be suspected as the causal agent. It is also possible that a strain from the turnip virus 1 group and one from the cauliflower-mosaic-virus group may both be present and induce a combined effect. Whether or not wild or cultivated plants in proximity may be infected from turnip will depend upon the strain of virus present. Obviously, in the case of some strains, cabbage and cauliflower, for instance, will not be infected. However, turnip is likely to be readily infected by any of the cruciferous strains that may be present in nearby plants if aphid vectors are available for its transfer.

SUMMARY

In this investigation four virus isolates from turnip (*Brassica rapa* L.), three collected at Madison, Wis., and one at Baton Rouge, La., have been studied in comparison with each other and with previously described viruses from cruciferous plants.

One of the isolates (T8) corresponds very closely in properties and in host range with the strain of turnip virus 1 described as cabbage virus A, and with the black ring and ring necrosis viruses from cabbage. The other three, T1, T6, and T9, are very similar to T8 in properties

and breadth of host range but differ from each other and from T8 in rather important host selectivity.   None of the last three infects cabbage, cauliflower, kale, brussels sprouts, or annual stock, though all infect green sprouting broccoli and only T9 infects kohlrabi and dames violet.

T8 and T9 infect all species of Nicotiana tested; T6, all but *N. rustica;* but T1 does not infect *N. tabacum, N. rustica, N. repanda,* or *N. sylvestris.*

All four infect petunia, chard, beet, and zinnia; all except T1 infect spinach.

It is pointed out that all viruses fully described thus far from crucifers are infectious to turnip.   The four isolates described here differ in one point or another from each other and from other viruses described. However, they are considered sufficiently similar to turnip virus 1 to be regarded as strains thereof and are distinct from the cauliflower-mosaic-virus group.

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EFFECTS OF PHOSPHORUS, NITROGEN, AND SOIL MOISTURE ON TOP-ROOT RATIOS OF INBRED AND HYBRID MAIZE [1]

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INTRODUCTION

That changes in environmental factors, such as moisture, nutrients, temperature, oxygen supply, and soil texture, can alter the weight, structure, and direction of growth of plants is common knowledge, yet few investigations have been made to determine how various environmental factors affect the balance of top-to-root growth of plants having different hereditary ratio potentials. The present study was made to determine the reaction of inbred lines and hybrids of maize (*Zea Mays* L.) having genetically different top-root ratios to three controlled environmental factors, namely, phosphorus, nitrogen, and soil moisture.

REVIEW OF LITERATURE

Boonstra (*3*),[2] using seven varieties of peas, found that while top-root ratios generally increase with increases in soil moisture and temperature, the reaction of the various varieties was different. Andrews (*1*) worked with two bush lima beans which differed in root-top ratio. A differential response was found between them with an increase in temperature, while their hybrid usually gave an intermediate response.

Harvey (*4*), studying the response of inbred and hybrid maize to ammonium nitrogen as compared with nitrate nitrogen, found significantly different top-root ratios between strains, but he failed to find any association between top-root ratios and the response of the strains to the different forms of nitrogen.

Smith (*8*), although not working on top-root ratios, noted differential responses among corn inbreds and hybrids on low phosphorus levels. Inbred lines efficient in utilization of low levels of phosphorus had a high ratio of secondary to primary roots, while inefficient lines had a low ratio. The F_1 generation was dominant for both phosphorus efficiency and the high ratio of secondary to primary roots. Increased root absorption due to the inheritance of branched type of root system was given as the explanation for phosphorus efficiency. Lyness (*6*), obtained similar results.

MATERIALS AND METHODS

One white flint and six yellow dent inbreds of maize and several of their F_1 hybrids were used. In previous tests (*7*) four of these

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[1] Received for publication September 14, 1943. Journal Paper No. J–1141 of the Iowa Agricultural Experiment Station. Project No. 250.
[2] Italic numbers in parentheses refer to Literature Cited, p. 377.

lines, KR, KR(Osf), Hy, and Ite had exhibited low top-root ratios while the other three, PR, R₄, and WF, had exhibited high ratios.[3]

In each test, plants were grown in the greenhouse from 6 to 8 weeks in completely randomized blocks. Water cultures were used in the phosphorus and nitrogen studies. A modification of the solution used by Smith (8) was employed in the phosphorus work, and the buffered solution recommended by Zinzadzé (10) for constant pH was used in the nitrogen studies. The solutions were made up as follows:

Modified Smith's solution		Buffered solution (Zinzadzé)	
Salt	*Grams per liter of solution*	*Salt*	*Grams per liter of solution*
KCl	0. 288	KCl	0. 286
$MgSO_4.7H_2O$	. 432	$MgSO_4.7H_2O$	. 500
$Ca(NO_3)_2.4H_2O$	. 469	NH_4NO_3	Varied
$(NH_4)_2SO_4$	. 115	$Ca_3(PO_4)_2$	. 500
KH_2PO_4	Varied		

The method of obtaining the colloidal buffer, $Ca_3(PO_4)_2$, used in Zinzadzé's solution has been described previously (7). Iron was added as needed in the form of ferric tartrate. Micronutrients were furnished by impurities in the salts and the tap water used for all cultures. Solutions were changed every 5 to 14 days, depending on the size of the plants. In the phosphorus work, as the solutions were made up, they were adjusted to pH 6.0 by the addition of N/10 H_2SO_4. Quart mason jars were used as containers throughout the water culture experiments.

Plants in the soil moisture studies were grown in glazed gallon jars filled with a compost-soil mixture. Calcium acid phosphate was added to the soil at the rate of 3 gm. per jar. Empty jar weights and dry soil weights were obtained. Field percentage and wilting percentage of the soil were determined according to the methods described by Loomis and Shull (5). These were used as criteria in adjusting soil moisture. When the plants were 10 days old a paraffined, 2½-inch clay pot was placed in an inverted position on the surface of each gallon jar and 1 inch of dry washed river sand was spread around the pot on top of the soil to reduce surface evaporation. The plants were watered through the hole in the inverted pot. Jar weight was corrected every 3 or 4 days for green plant weight. The jars were weighed daily in order to adjust for moisture when necessary.

Two plants served as a unit throughout. In harvesting, the roots of soil-grown plants were carefully washed. After harvesting, tops and roots were dried to a constant moisture level and weighed. Top-root ratios were calculated on these weights by dividing the dry weight of the top by the dry weight of the roots. Analyses of variance were carried out on the data obtained according to the method outlined by Snedecor (9).

EXPERIMENTAL RESULTS

INFLUENCE OF PHOSPHORUS CONCENTRATION ON INBRED AND HYBRID TOP-ROOT RATIOS

Test 1, conducted from January 25 to March 9, 1940, contained two high- and two low-ratio inbreds and four F₁ hybrids. Phosphorus con-

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[3] All lines were obtained through the courtesy of Dr. E. W. Lindstrom.

centrations of 5, 15, and 50 p.p.m. were established. In adjusting KH_2PO_4 to obtain the desired phosphorus supply, KCl was not adjusted to offset the changes in potassium concentration which automatically accompanied changes in the phosphorus level. Mean top-root ratios are presented in table 1 and the analysis of variance is given in table 2.

Lines, treatments, and their interaction were all highly significant. An increase in mean top-root ratios occurred when phosphorus was raised from 5 to 15 p.p.m. but no further increase was found for 50 p.p.m.

TABLE 1.—*Mean top-root ratios of inbreds and hybrids calculated from dry weights; test 1*

	Phosphorus concentration (p. p. m.)			
Lines	5	15	50	Mean
KR	2.00	2.41	2.47	2.29
KR×Hy	1.79	2.02	2.06	1.96
Hy	1.53	2.01	1.96	1.84
PR×Hy	1.92	2.26	2.42	2.20
PR	3.03	3.85	3.89	3.59
PR×R$_4$	2.37	3.10	2.93	2.80
R$_4$	2.21	2.80	2.88	2.63
KR×R$_4$	2.06	2.55	2.31	2.30
Mean	2.11	2.62	2.61	2.45

TABLE 2.—*Analysis of variance of top-root ratios; test 1*

Source of variation	Degrees of freedom	Mean square
Replications	6	0.29
Lines	7	6.55**
Treatments	2	4.79**
Lines×treatments	14	.12**
Error	138	.05
Total	167	--------------

**P less than 0.01.

One cause for the significant interaction of lines by treatments may be seen by comparing the mean differences between hybrids and their high- and low-ratio parents on each phosphorus concentration (table 3). The difference between the high-ratio parents and hybrids when grown on 15 p.p.m. was significantly larger than the corresponding difference on 5 p.p.m., being 0.85 ± 0.37. The same comparison with 50 and 5 p.p.m. gave a difference of 1.28 ± 0.37. The fact that these differences are positive in value indicates that top-root ratios for the high-ratio parents increased faster with increased phosphorus than did those for the hybrids. The same comparison between 50 and 15 p.p.m. was 0.43 ± 0.37. This, though nonsignificant, is positive in value, showing the same trend. Such differences between differences for any two phosphorus concentrations in the "Low-ratio parent minus hybrid" section of table 3 are negative, small, and nonsignificant, indicating that with increased phosphorus supply, hybrids and their low-ratio parents did not respond differently.

The differences between differences (interaction) for the high- and low-ratio inbreds for 15 and 5 p.p.m. of phosphorus were 0.68 ± 0.47

and for 50 and 5 p.p.m., 0.86 ± 0.47. These values are both positive, indicating that the top-root ratios increased with phosphorus concentration more rapidly for the high-than for the low-ratio parents. However, the values are not significant.

In this test each hybrid ratio tended to be nearer to that of its lower ratio parent than to that of its higher ratio parent. However, as a group the hybrid ratios were significantly larger than those of the lower ratio parents (-0.67 ± 0.15). This was also true at each individual phosphorus level (table 3).

TABLE 3.—*Mean differences in top-root ratios between hybrids and their high-ratio parents and between hybrids and their low-ratio parents; test 1*

HIGH-RATIO PARENT MINUS HYBRID

Hybrid	Phosphorus concentration (p. p. m.)			
	5	15	50	Mean
KR×Hy	+0. 21	+0. 39	+0. 41	+0. 34
PR×Hy	+1. 11	+1. 59	+1. 47	+1. 39
PR×R₄	+. 66	+. 75	+. 96	+. 79
KR×R₄	+. 15	+. 25	+. 57	+. 32
Sum	+2. 13	+2. 98	+3. 41	+2. 84
Standard error of sum	±. 26	±. 26	±. 26	±. 15

LOW-RATIO PARENT MINUS HYBRID

KR×Hy	−. 26	−. 01	−. 10	−. 12
PR×Hy	−. 39	−. 25	−. 46	−. 37
PR×R₄	−. 15	−. 30	−. 05	−. 17
KR×R₄	−. 06	−. 14	+. 16	−. 01
Sum	−. 86	−. 70	−. 45	−. 67
Standard error of sum	±. 26	±. 26	±. 26	±. 15

Test 2 was planned to check the results of test 1. Because of lack of seed, different inbred lines were used. Phosphorus concentrations were adjusted to 1.67, 5, and 15 p. p. m. Except for these changes the procedure was the same as for test 1. The test was conducted from May 13 to June 20, 1940. Table 4 presents the mean top-root ratios, table 5 the analysis of variance, and table 6 the mean differences between hybrids and their high- and low-ratio parents.

The treatment means show a continuous increase in top-root ratio with increased phosphorus concentration (table 4). As in test 1, the interaction of lines × treatments was highly significant, indicating that all lines did not respond the same to treatment. On breaking down the interaction, several individual comparisons were found to be significant. In the phosphorus concentration range from 1.67 to 5 and from 1.67 to 15 p. p. m. the high-ratio inbred parents of the hybrids increased in top-root ratio more than did the low-ratio parents of the hybrids. (The difference between two differences for each was 1.03 ± 0.52 and 1.92 ± 0.52, respectively.) No significant interactions were found between the hybrids and their low-ratio parents at the various phosphorus concentrations. When the hybrids and their high-ratio parents were compared on the various phosphorus concentrations it was found that values for the latter increased significantly more than the hybrid ratios when phos-

phorus was raised from 1.67 to 5 p.p.m. (1.09 ± 0.42) and from 1.67 to 15 p.p.m. (1.83 ± 0.42). These results confirm previous indications (test 1) that hybrids and their lower ratio parents do not, with increased phosphorus supply, increase in top-root ratio as rapidly as do the higher ratio parents.

In this test, ratios for hybrids were slightly smaller for each phosphorus concentration than were those of their lower ratio parents (table 6). However, none of the mean differences were significant.

TABLE 4.—*Mean top-root ratios of inbreds and hybrids calculated from dry weights; test 2*

Lines	Phosphorus concentration (p. p. m.)			
	1.67	5	15	Mean
KR (Osf)	1.61	2.11	2.38	2.03
KR (Osf)×ITE	1.58	1.70	2.22	1.83
ITE	1.93	1.99	2.28	2.07
ITE×PR	1.65	1.98	2.42	2.02
PR	2.29	3.30	4.02	3.20
PR×WF	2.25	2.60	3.15	2.67
WF	2.80	2.79	3.77	3.12
WF×KR (Osf)	2.08	2.26	2.72	2.35
Mean	2.02	2.34	2.87	2.41

TABLE 5.—*Analysis of variance of top-root ratios; test 2*

Source of variation	Degrees of freedom	Mean square
Replications	7	0.26
Lines	7	6.68**
Treatments	2	11.70**
Lines × treatments	14	.43**
Error	161	.07
Total	191	

**P less than 0.01.

TABLE 6.—*Mean differences in top-root ratios between hybrids and their high-ratio parents and between hybirds and their low-ratio parents; test 2*

HIGH RATIO PARENT MINUS HYBRID

Hybrid	Phosphorus concentration (p.p.m.)			Mean
	1.67	5	15	
KR (Osf)×ITE	+0.35	+0.29	+0.06	+0.23
ITE×PR	+.64	+1.32	+1.60	+1.19
PR×WF	+.04	+.70	+.87	+.54
WF×KR (Osf)	+.72	+.53	+1.05	+.77
Sum	+1.75	+2.84	+3.58	+2.73
Standard error of sum	±.30	±.30	±.30	±.17

LOW-RATIO PARENT MINUS HYBRID

KR (Osf)×ITE	+.03	+.41	+.16	+.20
ITE×PR	+.28	+.01	−.14	+.05
PR×WF	+.55	+.19	+.62	+.44
WF×KR (Osf)	−.47	−.15	−.34	−.32
Sum	+.39	+.46	+.30	+.37
Standard error of sum	±.30	±.30	±.30	±.17

INFLUENCE OF NITROGEN CONCENTRATION ON INBRED AND HYBRID TOP-ROOT RATIOS

Two tests were conducted to determine whether inbred and hybrid top-root ratios would respond to various concentrations of nitrogen in the same way that they had to phosphorus.

Test 3 was made with four inbreds, consisting of two low-ratio and two high-ratio lines, and four single crosses. Nitrogen levels of 5, 15, 45, and 135 p.p.m. were established. The plants were grown from December 26, 1940, to February 12, 1941. The mean top-root ratios are given in table 7, the analysis of variance in table 8, and the mean differences between hybrids and their high- and low ratio parents in table 9.

The treatment means increased steadily with nitrogen concentration. By subdividing the sum of squares for treatment into its linear, quadratic, and cubic parts, the mean squares obtained are 19.47, 0.87, and 0.25, respectively, showing that the bulk of the increase was linear.

In spite of the significant interaction of lines $\times$ treatments, no significant interaction between high- and low-ratio inbreds, between hybrids and their low-ratio parents, or between hybrids and their high-ratio parents was found. Apparently nitrogen caused no differential response among the three comparisons and the significance of the interaction variance was due to other causes.

In this test, hybrid ratios did not differ from those of the low-ratio parents, either for the test as a whole (-0.11 ± 0.12) or for any individual nitrogen level. They were significantly lower in all cases than those of the high-ratio parents.

TABLE 7.—*Mean top-root ratios of inbreds and hybrids calculated from dry weights; test 3*

Lines	Nitrogen concentration (p.p.m.)				Mean
	5	15	45	135	
KR (Osf)	1.20	1.38	1.60	2.25	1.60
Kr (Osf)$\times$R$_4$	1.65	1.78	2.01	2.59	2.01
R$_4$	2.44	2.77	2.78	[1] 2.97	2.74
R$_4\times$PR	1.98	2.27	2.59	3.00	2.46
PR	2.31	2.60	2.95	3.61	2.87
PR$\times$Hy	1.48	1.73	1.87	2.33	1.85
Hy	1.63	1.68	1.77	2.16	1.81
Hy$\times$KR (Osf)	1.20	1.40	1.55	2.09	1.56
Mean	1.74	1.95	2.14	2.62	2.11

[1] 1 plant unit lost by disease. Datum supplied by missing plot technique.

TABLE 8.—*Analysis of variance of top-root ratios; test 3*

Source of variation	Degrees of freedom	Mean square
Replications	5	0.16
Lines	7	6.24**
Treatments	3	6.87**
Lines$\times$treatments	21	.10**
Error	[1] 154	.03
Total	190	

**P less than 0.01.

[1] 1 degree of freedom subtracted for datum supplied by missing plot technique.

TABLE 9.—*Mean differences in top-root ratios between hybrids and their high-ratio parents and between hybrids and their low-ratio parents; test 3*

HIGH RATIO PARENT MINUS HYBRID

Hybrid	Nitrogen concentration (p.p.m.)				Mean
	5	15	45	135	
KR (Osf) × R₄	+0.79	+0.99	+0.77	+0.38	+0.73
R₄ × PR	+.33	+.33	+.36	+.61	+.41
PR × Hy	+.83	+.87	+1.08	+1.28	+1.01
Hy × KR (Osf)	+.43	+.28	+.22	+.07	+.25
Sum	+2.38	+2.47	+2.43	+2.34	+2.40
Standard error of sum	±.23	±.23	±.23	±.23	±.12

LOW RATIO PARENT MINUS HYBRID

	5	15	45	135	Mean
KR (Osf) × R₄	−.45	−.40	−.41	−.34	−.40
R₄ × PR	+.46	+.50	+.19	−.03	+.28
PR × Hy	+.15	−.05	−.10	−.17	−.04
Hy × KR (Osf)	.00	−.02	+.05	+.16	+.05
Sum	+.16	+.03	−.27	−.38	−.11
Standard error of sum	±.23	±.23	±.23	±.23	±.12

In test 4 the same lines were grown on the nitrogen levels of 4, 16, 64, and 256 p.p.m. As a precaution against toxicity from the ammonium ion of the ammonium nitrate, 0.5 p.p.m. of manganous chloride was added to the nutrient solution (2). The experiment was conducted from March 22 to April 30, 1941. Tables 10 and 11 present the data and analysis, respectively, and table 12, the mean differences.

The results of this test tend to substantiate the findings of the previous nitrogen experiment. Although the interaction of lines × treatments was again significant, no consistent interaction existed between the high- and low-ratio parents of the hybrids or between either group of parents and the hybrids.

Again hybrid ratios did not differ significantly from those of the low-ratio parents for any level of nitrogen except 64 p.p.m. No reason is known why this concentration should have caused a significant difference.

INFLUENCE OF SOIL MOISTURE ON INBRED AND HYBRID TOP-ROOT RATIOS

To determine how inbred and hybrid ratios respond to different concentrations of soil moisture, two tests were conducted in which the percentage of water in the soil was controlled.

In test 5, three inbreds and three hybrids were grown from October 15 to December 4, 1940, at moisture levels of 8–17, 11–17, and 24 percent. The field percentage of the soil used was 16.2, so it was necessary, for the first two groups, to successively add enough water to bring the soil moisture to 17 percent and then to allow the plants to reduce it to the lower moisture percentage before more was added. (The wilting percentage was 8.06). For the third level, jars were weighed daily and kept at 24 percent moisture. The results of the test are presented in tables 13, 14, and 15.

TABLE 10.—*Mean top-root ratios of inbreds and hybrids calculated from dry weights; test 4*

Lines	Nitrogen concentration (p.p.m.)				Mean
	4	16	64	256	
KR (Osf)	0.86	1.07	1.34	1.89	1.29
KR (Osf) × R₄	1.33	1.50	1.99	2.04	1.71
R₄	2.10	2.76	2.42	2.54	2.45
R₄ × PR	1.70	2.14	2.14	2.63	2.15
PR	2.04	2.39	2.68	3.25	2.59
PR × Hy	1.23	1.43	1.57	2.04	1.57
Hy	1.25	1.43	1.36	[1] 1.73	1.44
Hy × KR (Osf)	.92	1.08	1.30	1.55	1.21
Mean	1.43	1.72	1.85	2.21	1.80

[1] 1 plant unit lost by disease.   Datum supplied by missing plot technique.

TABLE 11.—*Analysis of variance of top-root ratios; test 4*

Source of variation	Degrees of freedom	Mean square
Replications	5	0.08
Lines	7	6.79**
Treatments	3	4.99**
Lines×treatments	21	.18**
Error	[1] 154	.02
Total	190	

[1] 1 degree of freedom subtracted for datum supplied by missing plot technique.
**P less than 0.01.

TABLE 12.—*Mean differences in top-root ratios between hybrids and their high-ratio parents and between hybrids and their low-ratio parents, test 4*

HIGH-RATIO PARENT MINUS HYBRID

Hybrid	Nitrogen concentration (p.p.m.)				Mean
	4	16	64	256	
KR (Osf)×R₄	+0.77	+1.26	+0.43	+0.50	+0.74
R₄×PR	+.34	+.25	+.54	+.62	+.44
PR×Hy	+.81	+.96	+1.11	+1.21	+1.02
Hy×KR (Osf)	+.33	+.35	+.06	+.18	+.23
Sum	+2.25	+2.82	+2.14	+2.51	+2.43
Standard error of sum	±.16	±.16	±.16	±.16	±.08

LOW-RATIO PARENT MINUS HYBRID

Hybrid	4	16	64	256	Mean
KR (Osf)×R₄	−.47	−.43	−.65	−.15	−.43
R₄×PR	+.40	+.62	+.28	−.09	+.30
PR×Hy	+.02	.00	−.21	−.31	−.12
Hy×KR (Osf)	−.06	−.01	+.04	+.34	+.08
Sum	−.11	+.18	−.54	−.21	−.17
Standard error of sum	±.16	±.16	±.16	±.16	±.08

TABLE 13.—*Mean top-root ratios of inbreds and hybrids calculated from dry weights; test 5*

Lines	Percent soil moisture			Mean
	8–17	11–17	24	
WF	2.91	3.47	5.60	3.99
WF×Hy	3.16	3.12	4.29	3.52
Hy	3.24	3.40	4.36	3.67
PR×Hy	3.47	3.53	4.21	3.74
PR	5.83	5.42	6.97	6.07
PR×WF	3.42	3.64	5.49	4.18
Mean	3.67	3.76	5.15	4.20

TABLE 14.—*Analysis of variance of top-root ratios; test 5*

Source of variation	Degrees of freedom	Mean square
Replications	5	0.17
Lines	5	16.19**
Treatments	2	24.83**
Lines×treatments	10	.95**
Error	85	.17
Total	107	

**P less than 0.01.

TABLE 15.—*Mean differences in top-root ratios between hybrids and their high-ratio parents and between hybrids and their low-ratio parents, test 5*

HIGH-RATIO PARENT MINUS HYBRID

Hybrid	Percent soil moisture			Mean
	8–17	11–17	24	
WF×Hy	−0.25	+0.35	+1.31	+0.47
PR×Hy	+2.36	+1.89	+2.76	+2.34
PR×WF	+2.41	+1.78	+1.48	+1.89
Sum	+4.52	+4.02	+5.55	+4.70
Standard error of sum	±.47	±.47	±.47	±.27

LOW-RATIO PARENT MINUS HYBRID

WF×Hy	+.08	+.28	+.07	+.14
PR×Hy	−.23	−.13	+.15	−.07
PR×WF	−.51	−.17	+.11	−.19
Sum	−.66	−.02	+.33	−.12
Standard error of sum	±.47	±.47	±.47	±.27

The mean top-root ratio for the lowest moisture level was 3.67, and for the intermediate level, 3.76. When these two means were compared they were found not to differ significantly, indicating that the effects of the treatment occurred between these levels and the high moisture level.

The interaction of lines×treatments was highly significant. No difference was found in the interaction of the high- and low-ratio hybrid parents with moisture levels. Nor was any interaction found

between the hybrids and their low-ratio parents. Therefore the significance of the interaction variance must have been caused by different reactions to treatment within the groups of inbreds and hybrids.

Mean differences between hybrid ratios and their low-ratio parents were nonsignificant, whether calculated for the experiment as a whole or for each individual moisture level.

Test 6 was similar to test 5 except that another moisture level was added and it was conducted at a different season of the year. The saturation percentage, not determined in the first test, and the field and wilting percentages of the soil used, were, respectively, 34.7, 14.5, and 7.0 percent. This test was conducted from April 23 to May 29, 1941. Tables 16, 17, and 18 present the results of the test.

TABLE 16.—*Mean top-root ratios of inbreds and hybrids calculated from dry weights; test 6*

Lines	Percent soil moisture				Mean
	7.5–15.5	11.0–15.5	17.5	20.8	
WF	1.99	2.57	2.68	3.41	2.66
WF×Hy	2.44	2.52	2.17	2.71	2.46
Hy	2.15	2.85	2.80	3.09	2.72
PR×Hy	2.41	2.71	2.71	2.75	2.64
PR	3.42	3.68	4.15	4.98	4.06
PR×WF	2.40	2.65	2.80	3.46	2.83
Mean	2.47	2.83	2.88	3.40	2.90

TABLE 17.—*Analysis of variance of top-root ratios: test 6*

Source of variation	Degrees of freedom	Mean square
Replications	4	0.46
Lines	5	6.77**
Treatments	3	4.42**
Lines×treatments	15	.37**
Error	92	.06
Total	119	....

**P less than 0.01.

TABLE 18.—*Mean differences in top-root ratios between hybrids and their high-ratio parents and between hybrids and their low-ratio parents; test 6*

HIGH-RATIO PARENT MINUS HYBRID

Hybrid	Percent soil moisture				Mean
	7.5–15.5	11.0–15.5	17.5	20.8	
WF×Hy	−0.29	+0.33	+0.63	+0.38	+0.26
PR×Hy	+1.01	+.97	+1.44	+2.23	+1.41
PR×WF	+1.02	+1.03	+1.35	+1.52	+1.23
Sum	+1.74	+2.33	+3.42	+4.13	+2.90
Standard error of sum	±.32	±.32	±.32	±.32	±.16

LOW-RATIO PARENT MINUS HYBRID

WF×Hy	−.45	+.05	+.51	+.70	+.20
PR×Hy	−.26	+.14	+.09	+.34	+.08
PR×WF	−.41	−.08	−.12	−.05	−.17
Sum	−1.12	+.11	+.48	+.99	+.11
Standard error of sum	±.32	±.32	±.32	±.32	±.16

The results were similar to those of test 5. While interaction of lines × treatments was highly significant, no interaction was found between moisture levels and the high- and low-ratio lines used as hybrid parents. Hybrids differed from their low-ratio parents in their reaction to soil moisture only when comparisons were made involving the lowest moisture level. The mean difference between the low-ratio parents and hybrids at the low moisture level was −1.65±0.37 less than the average mean difference between low inbreds and hybrids for the other three moisture levels. The same comparison of differences for the hybrids and their high-ratio parents was −1.55±0.37. These values are highly significant. Why the reaction of hybrids and inbreds at this soil-moisture level should differ from that at the other levels is not apparent.

No differences were found between the total mean top-root ratios of the hybrids and those of the low-ratio parents. When compared on the basis of individual moisture percentages they differed only on the lowest level, the hybrids having a larger ratio than the inbreds.

DISCUSSION

Top-root ratios increased for both hybrids and their parental inbreds as phosphorous was raised from limiting to optimum concentrations. No significant differences in rate of increase between the hybrids and their lower ratio parents occurred. When rate of hybrid increase was compared with that of the higher ratio parents, it was found that, as the phosphorus level was raised, ratios for these inbreds increased faster than those for the hybrids; in other words, as phosphorus became limiting, top-root ratios decreased more rapidly for the high-ratio parents than for the hybrids or their low-ratio parents.

As previously stated, Smith (8) found a high ratio of secondary to primary roots in phosphorus-efficient inbred lines of corn and a low ratio with phosphorus-inefficient lines. He advanced the theory that phosphorus efficiency lies in a large absorbing surface, a factor of importance to the plant since phosphorus moves very little in the soil solution. Hence, to grow well on soils low in phosphorus the plant needs a ramifying root system. In the nutrient solution used, in which phosphorus becomes quickly precipitated out of solution, the plant with the larger absorptive area would have the advantage in absorbing phosphorus before it became unavailable.

When the quantity of any substance absorbed by the roots, i. e., any nutrient or soil moisture, is insufficient for optimum growth, the largest part of that absorbed is used (along with organic foods translocated from the tops) in root growth. Top growth is thereby relatively more limited than root growth, resulting in low top-root ratios. Inversely, when the amount of the substance absorbed by the roots is greater, relatively larger quantities are translocated to the tops, where it is utilized in top growth. Under these conditions most of the organic foods produced in the tops are utilized in their growth and very little is translocated to the roots, which results in larger top-root ratios.

Therefore, if a correlation between root weight and root-absorbing surface exists, plants with the lowest top-root ratio (i. e., with the most roots and therefore with the greatest absorbing surface per unit of

top weight) will absorb the most phosphorus per weight of top, expecially if phosphorus is low, and the result will be less phosphorus deficiency and less reduction in top growth. This in turn would lead to a smaller reduction in top-root ratios, or, inversely, to smaller increases with added phosphorus, when the initial supply is limited. Hybrids with top-root ratios similar to those of their low-ratio parents would respond like their parents. The results of both tests 1 and 2 confirm this interpretation.

Hybrid and inbred ratios responded similarly to various nitrogen levels, the rate of ratio increase as nitrogen was raised from a low to a high concentration being similar for hybrids, for their low-ratio parents, and for their high-ratio parents. This would be expected since nitrogen is not precipitated out of solution in water cultures and should be equally available to all plants regardless of the type of their root system.

Hybrid and inbred top-root ratios increased similarly as soil moisture was raised from low to high levels. Although the water supply may be reduced below that necessary for optimum growth, causing lowered top-root ratios (tops suffering the most, being farthest from the supply), roots will continue to grow throughout the soil, obtaining moisture until it is reduced to a point where it is comparatively unavailable (approximately the wilting percentage). If soil moisture is reduced to the same level for all plants before being replenished, there is no reason to assume that the top-root ratio decrease would be proportionally more for a plant with a large ratio (i. e., few roots) than for a plant with a small ratio.

The results of these tests add weight to information already reported (7) that hybrid top-root ratios either did not differ or differed only slightly from those of their lower ratio parents. This was true regardless of whether the hybrid consisted of two high-, two low-, or a high- and a low-ratio inbred.

SUMMARY

Studies were made to determine the top-root ratios of maize hybrids, their low-ratio parents, and their high-ratio parents at different controlled concentrations of phosphorus, nitrogen, and soil moisture. In each experiment three or four concentrations were used, ranging from those definitely limiting, to those ample for good plant growth.

Top-root ratios increased with phosphorus concentration, but did not increase as rapidly (measured arithmetically) for hybrids or their low-ratio parents as for their high-ratio parents. No difference in rate of ratio increase was found between hybrids and their low-ratio parents.

The explanation is advanced that hybrids and their low-ratio parents, having more roots per unit of top weight than the high-ratio parents, absorb proportionally more phosphorus before it is precipitated out of the nutrient solution, and therefore suffer less phosphorus starvation when the phosphorus supply is low. This would result in less reduction in top-root ratio for the hybrids and low-ratio lines when grown on limited supplies of phosphorus and consequently a relatively smaller increase with additional phosphorus.

Top-root ratios increased with an increase in nitrogen concentration but no differential rate of arithmetic increase was found between

hybrids and either their high- or low-ratio parents. The results when soil moisture was increased were similar to those with nitrogen.

In all tests F_1 hybrid ratios either did not differ or differed only slightly from those of their lower ratio parents.

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VIRUSES ASSOCIATED WITH CABBAGE MOSAIC [1]

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INTRODUCTION

A mosaic disease of cabbage (*Brassica oleracea* var. *capitata* L.) in southeastern Wisconsin was described by Larson and Walker in 1939.[3] The host range and certain properties of the infectious virus also were detailed. In 1937 Tompkins [4] gave an account of a mosaic disease of cauliflower (*B. oleracea* var. *botrytis* L.) in California, and in 1938 Tompkins, Gardner, and Thomas [5] described black ring, a virus disease of cabbage in the same State. In 1941 Larson and Walker [6] described another virus disease of cabbage, ring necrosis, in Wisconsin.

Further studies of the cabbage mosaic described earlier by Larson and Walker [3] have revealed that, as a rule, two distinct viruses (referred to herein as viruses A and B) occur systemically in infected plants. The present paper is an account of the separation of the two viruses, their properties, and the symptoms which they produce on various hosts independently and in combination. The cabbage black ring virus and the cauliflower mosaic virus of Tompkins [4] were studied comparatively in the same environment.

MATERIALS AND METHODS

The studies of symptoms, host range, and physical properties of the viruses were carried out in greenhouses which were fumigated frequently to keep them free from insects. Stock cultures of the viruses were carried in plants growing in specially caged insectproof compartments. Plants used for experiments were grown in 4-inch clay pots at temperatures suitable for their normal development. Mechanical inoculations with juice extracted from stock-virus plants were made by rubbing with cotton or cheesecloth over the surface of one or two lower leaves on which finely powdered carborundum had been sprinkled previously. Aphid transmission was carried out by transferring adult insects from virus-free nurture cabbage plants to the stock-virus plant and allowing them to feed for 48 hours or more. Aphids from the infected plant were transferred to the test plant by means of a camel's

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hair brush, contact between the brush and the test plant being avoided. Sometimes a leaf with aphids was detached from the stock plant and placed on a small piece of paper and the paper was allowed to rest on a leaf of the test plant until the aphids had migrated to the new plant.

Upon the appearance of symptoms in a given test plant, the presence of the virus concerned was confirmed by mechanical transfer to cabbage or to tobacco (*Nicotiana tabacum* L.) when the latter was known to give a characteristic symptom. In the case of hosts within the Chenopodiaceae and Amaranthaceae it was learned that some constituent of the extracted juice of the host inactivated the virus and the presence of the virus was thus obscured. In such instances recovery was made only by means of insect vectors.

Physical properties of the viruses were studied with extracts from cabbage plants (var. Jersey Queen) infected with the virus or viruses in question. Infected plants were triturated with a sterile mortar and pestle. The crude extract was filtered through 8 thicknesses of cheesecloth without any application of pressure. For tests of tolerance to dilution the original extract was diluted with 9 parts of sterile distilled water in the case of virus B and with 99 parts in the case of virus A. This mixture was used in the preparation of each higher dilution tested. Dilutions were made in quantities of 100 cc. and shaken vigorously for 2 to 3 minutes, when a 10-cc. aliquot was drawn for the inoculation. A single pipette was used for each transfer; the inoculation from the highest dilution was made first, and successively lower dilutions were taken in order. For thermal inactivation determinations the filtered, undiluted extract was drawn into thin-walled, 2-mm. glass tubes which were sealed in a flame at one end and plugged with cotton at the other. The tubes were submerged in a circulating water bath for a 10-minute period at the desired temperature. The temperature was kept constant within $\pm 0.1°C$. At the end of the period the sealed end of the tube was broken and the heated extract forced into a sterile mortar from which the inoculation was made. Determination of longevity in vitro was made by storing the filtered, undiluted extract in test tubes plugged with cotton in a constant-temperature incubator at 20°. A single test tube was removed at each stipulated interval and the contents used as inoculum without dilution. All glassware was sterilized before use. For cabbage virus A and the black ring virus, tobacco plants (var. Connecticut Havana No. 38) were used as test plants and determinations were made by counting the lesions on 2 leaves of each of 10 plants in each test. The same method was used for determining virus A in a mixture containing viruses A and B. For cabbage virus B and the cauliflower mosaic virus, 10 cabbage plants (var. Jersey Queen) per test were used.

Stock cultures of cauliflower mosaic virus and black ring virus were obtained from C. M. Tompkins, of the California Agricultural Experiment Station. The viruses of cabbage mosaic were obtained in southeastern Wisconsin. Certain comparative isolates [7] were collected from other parts of the United States.

SEPARATION OF THE TWO CABBAGE VIRUSES

In the original study of cabbage mosaic [8] it was found that the infectious entity was readily transferred to a wide host range. On

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[7] The term "isolate" is used in this paper to designate a virus from a given source without implying that it is necessarily a distinct strain.
[8] See footnote 3.

tobacco systemic infection did not occur but local necrotic lesions up to 3 mm. in diameter were produced. In determinations of longevity in vitro, tolerance to dilution, and the point of thermal inactivation the local-lesion method of determining the activity of the virus extract was used. The thermal inactivation point determined by this method was 55° C. In later studies it was found that when the virus extract was heated several degrees higher than 55° it was still infectious on cabbage. Moreover, on cabbage plants so infected, symptoms differed from those which developed after inoculation with the original extract. This virus, hereafter referred to in this paper as cabbage virus B, produced symptoms not unlike an isolate obtained in the course of the earlier investigation by Larson and Walker [8] and set aside for further study.

Virus B was found to affect all cruciferous species earlier found susceptible to cabbage mosaic [8] except hoary alyssum (*Berteroa incana* (L.) DC.) and dames violet (*Hesperis matronalis* L.). It failed, however, to produce symptoms on any noncruciferous plant which had previously been found to react to the cabbage mosaic extract.

TABLE 1.—*Properties of cabbage virus A and black ring virus as determined by the number of local lesions formed on inoculated tobacco*

[2 leaves on each of 10 plants inoculated in each trial]

Type and degree of treatment	Cabbage virus A				Cabbage viruses A and B	Black ring virus
	First trial	Second trial	Third trial	Fourth trial		
Dilution:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
0	2,176	2,151	1,150	4,330	2,366	5,260
1–100	472	33	205	1,749	592	3,401
1–1,000	50	14	20	114	150	278
1–1,500	20	17	11	47	65	190
1–2,000	6	13	1	31	18	57
1–2,500	12	15	2	26	14	74
1–3,000	2	21	8	24	11	61
1–4,000	0	2	0	18	9	46
1–5,000	0	0	3	17	1	48
1–10,000	0	0	0	0	0	6
1–15,000				0	0	0
Heating for 10 minutes (° C.):						
None	421	2,151	494	501	2,128	2,458
51	232	1,111	139	412	776	1,620
52	209	917	111	350	314	1,350
53	183	896	87	279	222	250
54	180	681	72	179	180	144
55	170	486	29	86	58	158
56	60	468	3	44	20	28
57	18	65	3	7	18	24
58	7	19	1	1	26	2
59	0	6	0	1	4	0
60	0	0	0	0	0	0
61	0	0	0	0	0	0
Aging in vitro at 20° C.:						
None	2,842	3,110	2,430		2,260	3,990
1 day	2,460	2,980	1,760		2,012	3,484
2 days	1,606	1,700	136		1,050	2,905
3 days	580	538	8		202	314
4 days	468	410	3		17	54
5 days	472	286	6		4	4
6 days	406	136	7		10	2
7 days	164	0	4		4	0
8 days	0	0	0		0	0
9 days	0	0	0		0	0
10 days	0	0	0		0	0

[8] See footnote 3.

When noncruciferous plants were inoculated with a cabbage mosaic extract symptoms previously described [8] developed. However, when extracts from such plants were inoculated into cabbage, symptoms on the latter differed in certain respects, the virus thus obtained being free from cabbage virus B. The former is referred to hereafter in this paper as cabbage virus A.

PROPERTIES OF CABBAGE VIRUSES A AND B

When viruses A and B had been separated by the method described above they were carried in stock-virus plants for further study. Virus A was carried in plants of *Nicotiana glutinosa* L. The fact that this

TABLE 2.—*Properties of cabbage virus B and cauliflower mosaic virus as determined by the number of inoculated cabbage plants that became infected*

[10 plants inoculated in each trial]

Type and degree of treatment	Plants infected with—				
	Cabbage virus B				Cauliflower mosaic virus
	First trial	Second trial	Third trial	Fourth trial	
	Number	*Number*	*Number*	*Number*	*Number*
Dilution:					
0	10	10	10	10	10
1-10	9	6	7	10	10
1-50	6	4	4	8	9
1-100	5	1	3	5	8
1-500	2	0	0	1	5
1-1,000	2	0	0	1	2
1-1,500	0	0	0	0	1
1-2,000	0	0	0	0	0
1-3,000				0	0
Heating for 10 minutes (° C.):					
None	9	10	10	10	10
56	7	9	2	7	8
58	5	8	2	9	10
60	3	6	4	7	8
62	3	8	2	9	7
64	4	7	2	9	9
66	4	6	7	9	7
68	3	5	2	7	6
70	0	7	5	6	5
72	1	4	2	6	6
74	0	2	1	3	4
76	0	0	0	1	2
78	0	0	0	0	0
Aging in vitro at 20° C.:					
None	10	10	10	9	10
1 day	7	8	9	4	10
2 days	3	4	8	6	9
3 days	1	2	5	6	10
4 days	0	1	2	2	8
5 days	0	0	1	1	7
6 days	0	0	0	0	6
7 days	0	0	0	0	3
8 days	0	0	0	0	3
9 days	0	0	0	0	2
10 days	0	0	0	0	1
11 days	0	0	0	0	0
12 days	0	0	0	0	0
13 days	0	0	0	0	0
14 days	0	0	0	0	0
15 days	0	0	0	0	0

host was immune to virus B insured against chance contamination by the latter. Virus B was carried in cabbage plants. In order to insure against chance contamination with virus A, the extract was frequently

[8] See footnote 3.

heated to 65° C. before being transferred to new stock-virus plants.

The thermal inactivation points, the tolerance to dilution, and the longevity in vitro of the two viruses were determined by methods already described. The results are given in tables 1 and 2.

Virus A was inactivated by dilution at 1–4,000 in one trial, at 1–5,000 in one trial, and at 1–10,000 in two trials. Virus B was inactivated at 1–500 in two trials and 1–1,500 in two trials. Thus virus A was distinctly the more tolerant to dilution. Virus A was inactivated at 59° C. in two trials and at 60° in two trials, while virus B was inactivated at 74° in one trial, at 76° in two trials, and at 78° in one trial. Thus virus B was distinctly the more tolerant to heat. Virus A was inactivated in vitro at 20° at 7 days in one trial and at 8 days in two trials, while virus B was inactivated at 4 days in one trial, at 5 days in one trial, and at 6 days in two trials. Thus virus A was definitely more resistant to aging than virus B.

If the properties of cabbage virus A are compared with those of the cabbage mosaic virus described by Larson and Walker [9] on the basis of reaction on tobacco, some degree of resemblance is found. The cabbage mosaic virus preparation, which, it is now presumed, was a mixture of viruses A and B, was inactivated by dilution to 1–2,000, by heating at 55° C. for 10 minutes, and by aging at 20° to 22° for 72 hours. In each case it was somewhat less tolerant to the treatment than was cabbage virus A. When an extract taken from plants infected with both A and B viruses was used on tobacco, as shown in table 1, the results were very similar to those obtained with virus A alone. Whether the differences are very significant is questionable. Judging from the number of local lesions per leaf caused by the untreated extracts, the concentration of virus A in the cabbage mosaic preparation used by Larson and Walker [9] was much less than that in the extracts used for the present cabbage virus A determinations. This may account for some of the discrepancy between the two determinations.

In the tests of Tompkins, Gardner, and Thomas, [10] cabbage black ring virus was inactivated by heat at 59° C., by dilution at 1–1,000, and in vitro at 3 days. When a culture obtained from Tompkins was run concurrently with the tests reported here, as shown in table 1, inactivation by heat was at 59°, by dilution at 1–15,000, and by aging at 7 days. Allowing for discrepancies due to differences in handling and methods, it is obvious that cabbage virus A and the black ring virus were very similar in the physical properties studied.

Of the crucifer viruses previously described, cauliflower mosaic virus [11] conformed most closely in properties, symptoms, and host range to cabbage virus B. The former was reported by Tompkins as inactivated by dilution at 1–3,000, by heat at 75° C., and in vitro after aging 15 days at 22°. When a culture of cauliflower mosaic virus obtained from Tompkins was tested concurrently with virus B, it was found it was inactivated by dilution at 1–2,000, by heat at 78°, and by aging at 20° at 11 days (table 2). Except for reaction to aging, the two viruses were very close to each other in the physical properties studied.

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[9] See footnote 3.
[10] See footnote 5.
[11] See footnote 4.

SYMPTOMS AND HOST RANGE

It is obvious that the symptoms on various hosts described for cabbage mosaic by Larson and Walker[12] were concerned with the reaction of the two viruses, cabbage viruses A and B, when cruciferous hosts were involved. When noncruciferous hosts were used, only cabbage virus A was concerned, since in this investigation cabbage virus B was not found to infect any but cruciferous plants. The assumption that the cabbage virus previously described[12] is a mixture of cabbage viruses A and B is based on the fact that in practically every case in which cabbage mosaic has been collected during midseason in southeastern Wisconsin and in the Puget Sound cabbage-seed-growing area of western Washington both viruses have been found to be present. In the cool weather of late autumn in Wisconsin virus A declines in amount. In view of the circumstances just cited, it was considered advisable to restudy the effect of the two viruses separately and together on the same list of hosts previously recorded. Furthermore, in view of the possible relation of viruses A and B both to the black ring virus and to the cauliflower mosaic virus, these too were included in the studies.

It became evident early in the work that cabbage virus A was favored by relatively high air temperatures, while cabbage virus B was favored by relatively low air temperatures. Therefore the host-range studies were conducted simultaneously in a greenhouse controlled at 28° C. and in one at 20°, and each species was inoculated simultaneously with all viruses.

HOST RANGE OF THE VIRUSES

The symptoms on cruciferous hosts in response to each virus and to viruses A and B in combination are given in table 3. It is to be noted that all four viruses were infectious to all cruciferous species tested except yellow rocket, hoary alyssum, dames violet, and Virginia stock. The last three mentioned were infected by cabbage virus A and the black ring virus but not by cabbage virus B and the cauliflower mosaic virus, and the first was not infected by any of them.

The cauliflower mosaic virus and cabbage virus B infected no noncruciferous plants, while cabbage virus A and the black ring virus infected many such hosts belonging to numerous genera and families (table 4). When viruses A and B in combination were inoculated to susceptible noncruciferous hosts, the symptoms were identical with those produced when virus A alone was used.

Over this wide range of cruciferous and noncruciferous hosts both virus A and the black ring virus were found to be infectious on all species susceptible to either and tested with both, with the exception of *Solanum integrifolium*, which was not infected by the black ring virus. Neither virus infected by the mechanical method Jerusalem-cherry, bittersweet, jimsonweed, belladonna, cherry pepper, hyacinth-bean, scarlet runner bean, soybean, cowpea, morning-glory, okra, four-o'clock, knapweed, French marigold, yellow rocket, or sweet alyssum.

Thus it is apparent that cabbage virus A and black ring virus are very similar in their host range. Likewise virus B and the cauliflower mosaic virus are similar in host range and are both confined to hosts within the Cruciferae.

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[12] See footnote 3.

TABLE 3.—*Symptoms produced on young plants of various species of Cruciferae in the greenhouse at constant temperatures of 20° and 28° C. when inoculated mechanically with cabbage virus A, cabbage black ring virus, cabbage virus B, cauliflower mosaic virus, and cabbage viruses A and B together*

Scientific and common names	Symptoms resulting from inoculation with—				
	Cabbage virus A	Black ring virus	Cabbage virus B	Cauliflower mosaic virus	Cabbage viruses A and B
Brassica oleracea var. *capitata* L. (cabbage, var. Jersey Queen).	Local: None. Systemic: At 28° C. mild chlorotic mottle, with or without vein clearing, rapidly becoming severe and diffuse until mostly chlorotic with small dark-green islands; leaf-bloom reduction moderate; petioles somewhat elongated; severe leaf malformation; premature leaf abscission; no necrosis; severe stunting. At 20° young leaves symptomless or with occasional chlorotic spots; chlorotic lesions more distinct than at 28°; mild mottle; slight necrosis; slight stunting.	Local: None. Systemic: Similar to virus A but milder. At 28° C. mottle finer and less severe; vein clearing uncommon, stunting, distortion, and leaf-bloom reduction less than for virus A; round, dark-green spots with chlorotic halos or dark-green rings bounded on either side by light-green zones; no necrosis. At 20° rings and ring spots more conspicuous than with virus A; moderate to severe necrosis as black spots and black rings; mottle milder than at 28°.	Local: None. Systemic: At 28° C. mild vein clearing increasing until prominent, then receding until barely discernible; mild diffuse mottle; leaf-bloom reduction moderate, decreasing with age; no necrosis; no distortion; symptoms becoming masked in time; slight stunting. At 20° vein clearing slower but similar, becoming prominent sometimes as chlorotic vein banding; mild mottle; no stunting; enations sporadic; symptoms less pronounced at 28° but never completely masked as at 28°.	Local: None. Systemic: Similar to virus B but. always milder.	Local: None. Systemic: At 28° C. at first same as virus A; vein clearing becoming prominent and mottle more severe but of much finer type than with virus A, leaves taking on a yellow-white speckled appearance; severe necrosis as streaking of veins and blue-black sunken flecks in parenchyma; severe stunting. At 20° mottle milder, vein clearing more prominent and more persistent than at 28°; chlorotic vein banding common; necrosis, stunting, leaf-bloom reduction milder than at 28° but more severe than with virus A at 20°.
B. oleracea var. *botrytis* L. (cauliflower, var. Early Snowball).	Local: None at 28°. Sporadic irregular necrotic pattern at 20°. Systemic: At 28° coarse chlorotic mottle, occasional vein clearing; midrib curvature; unilateral leaf development on some plants; very mild purplish rings at margins of chlorotic spots on lower side of leaf, causing various patterns; mild stunting. At 20° mild vein clearing; mild mottle; very slight stunting; purple rings much more prominent than at 28°.	Local: None at 28°. At 20° prominent chlorotic rings and ring spots common, becoming necrotic with age. Systemic: At 28° similar to virus A, but mottle milder, chlorotic lesions more distinct, less distortion, slightly more stunting. At 20° ring necrosis much more severe than at 28° and than virus A at 20°; on young leaves numerous but small whitish rings developing on upper surface.	Local: None. Systemic: At 28° vein clearing decreasing, diffuse mottle increasing with age; characteristic midrib curvature; mild stunting. At 20° pronounced vein clearing and vein banding; mild diffuse mottle; midrib curvature; very mild stunting; no necrosis.	Local: None. Systemic: Symptoms similar to virus B but decidedly more pronounced.	Local: None. Systemic: At 28° Initial symptoms identical with those of virus A but vein clearing of virus B becoming more prominent and mottle more severe; leaf malformation and stunting more severe than with virus A. At 20° similar to 28° with milder mottle and stunting but more pronounced vein clearing and necrotic rings.

TABLE 3.—*Symptoms produced on young plants of various species of Cruciferae in the greenhouse at constant temperatures of 20° and 28° C. when inoculated mechanically with cabbage virus A, cabbage black ring virus, cabbage virus B, cauliflower mosaic virus, and cabbage viruses A and B together*—Continued

Scientific and common names	Symptoms resulting from inoculation with—				
	Cabbage virus A	Black ring virus	Cabbage virus B	Cauliflower mosaic virus	Cabbage viruses A and B
B. oleracea var. *botrytis* L. (broccoli, var. Italian Green Sprouting).	Local: None. Systemic: At 28° similar to cabbage except slight stunting and leaf malformation; no premature defoliation. At 20° mottle less diffuse; vein clearing more prominent; stunting very mild.	Local: None at 28°. At 20° small chlorotic spots later forming an irregular necrotic pattern. Systemic: At 28° coarse chlorotic mottle much as in cabbage; no necrosis. At 20° mottle milder but necrosis developing with age; distinctly more necrotic than with virus A.	Local: None. Systemic: At 28° similar to cabbage with mild stunting. At 20° vein clearing and fine mottle; stunting less than at 28°.	Local: None. Systemic: Similar to but milder than virus B at first, gradually becoming indistinguishable from virus B.	Local: None. Systemic: At 28° similar to cabbage. No necrotic spots or patterns as described by Larson and Walker,[1] possibly because plants were discarded too soon.
B. oleracea var. *gongylodes* L. (kohlrabi, var. Early White Vienna).	Local: None. Systemic: At 28° similar to cabbage except less malformation and stunting and formation of necrotic rings or spots conspicuous on older leaves. At 20° chlorotic mottle; some streak along veins; premature leaf abscission. No evidence of reduction in size of storage organ.	Local: None at 28°. At 20° irregular necrotic pattern on inoculated leaves. Systemic: At 28° numerous chlorotic rings, later giving rise to a coarse mottle as in cabbage; no necrosis; symptoms milder than on cabbage. At 20° numerous small chlorotic rings giving a marble mosaic effect; necrotic ringing increasing with age; very similar to virus A.	Local: None. Systemic: At 28° same type as for cabbage but very mild. At 20° similar to cabbage, more severe than at 28° but still very mild, slightly more pronounced than those of cauliflower mosaic virus.	Local: None. Systemic: Of same type as virus B but slightly less conspicuous.	Local: None. Systemic: At 28° similar to cabbage; mottle more diffuse and chlorosis more pronounced than for virus A; scattered necrotic spots; symptoms generally quite mild. At 20° fairly mild diffuse mottle; conspicuous vein clearing.
B. oleracea var. *viridis* L. (kale, var. Dwarf Green Curled).	Local: Necrosis on inoculated leaves of some plants. Systemic: At 28° similar to cabbage with irregular chlorotic blotches quite extensive; stunting very slight. At 20° similar to 28° except chlorotic blotches more distinct.	Local: None at 28°. At 20° necrosis as an irregular pattern. Systemic: At 28° mild but coarse chlorotic mottle; no necrosis; slight stunting. At 20° mottle is very mild; necrosis becoming conspicuous with age; no chlorotic blotches as for virus A.	Local: None. Systemic: At 28° similar to cabbage. At 20° similar to cabbage except fleck necrosis on some plants.	Local: None. Systemic: Similar to virus B.	Local: None. Systemic: At 28° similar to cabbage except streak on veins common; symptoms relatively mild; stunting mild. At 20° similar to 28° except necrosis quite severe.

[1] See footnote 3.

B. oleracea var. *gemmifera* DC. (brussels sprouts, var. Long Island Mammoth).	Local: None at 28°. At 20° scattered chlorotic lesions. Systemic: At 28° similar to but milder and slower than on cabbage; streak necrosis. At 20° similar to cabbage except necrotic rings similar to cauliflower developing after 3 to 4 weeks.	Local: None at 28°. At 20° severe necrosis causing entire leaf to die. Systemic: At 28° indistinguishable from virus A. At 20° few chlorotic lesions, rapidly giving way to a severe systemic necrosis; markedly different from virus A at 20°.	Local: None. Systemic: Similar to cabbage, very mild at 28°; vein clearing prominent at 20°.	Local: None. Systemic: Very similar to those of virus B.	Local: None. Systemic: At 28° similar to but more severe than virus A. At 20° vein clearing and vein banding more conspicuous than at 28°.
B. rapa L. (turnip, var. Purple Top White Globe).	Local: None. Systemic: At 28° vein clearing; diffuse mottle becoming severe; marked leaf malformation; severe stunting; root and hypocotyl enlargement almost completely inhibited; highly lethal, killing majority of plants before appearance of symptoms. At 20° symptoms milder throughout.	Not tested.	Local: None. Systemic: At 28° slower than virus A; temporary vein clearing; severe mottle and chlorosis; unilateral leaf distortion; severe leaf stunting and savoying; root and hypocotyl enlargement suppressed; lethal. At 20° mild vein clearing for extended period followed later by severe mottle; symptoms more severe than at 28°; lethal.	Local: None. Systemic: Similar to those for virus B except more severe early; later virus B symptoms reached identical stage.	Local: None. Systemic: At 28° most plants killed outright; others showed more diffuse mottle than virus A and more pronounced vein clearing. At 20° more prominent vein clearing, more diffuse mottle, and more severe stunting than virus A.
B. campestris L. (wild yellow mustard).	Local: At 28° local lesions and severe necrosis on old plants. Systemic: At 28° vein clearing; chlorotic mottle; severe stunting; highly lethal; severe necrosis on old plants. At 20° vein clearing and mottle; severe stunting; highly lethal.	Local: As for virus A. Systemic: As for virus A except less necrosis at 28°. Severe stunting, chlorosis, vein clearing and leaf distortion; death occurs later than for virus A. At 20° very similar to virus A except slightly more severe.	Local: None. Systemic: At 28° prominent vein clearing, followed tardily by midrib curvature and necrotic flecking; moderate stunting. At 20° vein clearing and general chlorosis; stunting more severe than at 28°. Not lethal at 28° or 20°.	Local: None. Systemic: Similar to virus B but more rapid and severe; highly lethal.	Local: None. Systemic: At 28° vein clearing and diffuse mottle, former increasing in prominence; unilateral leaf malformation; severe stunting; highly lethal. At 20° similar to 28° but milder.
B. hirta Moench (*B. alba* (L.) Rabenh. (white mustard).	Local: None. Systemic: At 28° young leaves rolled as if burnt; mottle of green and whitish areas; rapid necrosis spreading along veins; highly lethal. At 20° similar to 28°.	Local: None. Systemic: Severe chlorotic mottle and vein clearing but no necrosis; stunting and leaf distortion very severe. Plants not dead in 3 weeks after inoculation as for virus A. At 20° symptoms slightly more severe than at 28°.	Local: None. Systemic: At 28° vein clearing; chlorotic spots; stunted, curled leaves; tan necrotic spots; severe stunting; killing of growing point; highly lethal. At 20° mild mottle and distortion, vein clearing, rosetting; highly lethal.	Local: None. Systemic: Similar to virus B but more pronounced, more stunting; marked rosetting; very highly lethal.	Local: None. Systemic: At 28° similar to virus A; at 20° similar to virus A but more severe.

TABLE 3.—*Symptoms produced on young plants of various species of Cruciferae in the greenhouse at constant temperatures of 20° and 28° C. when inoculated mechanically with cabbage virus A, cabbage black ring virus, cabbage virus B, cauliflower mosaic virus, and cabbage viruses A and B together*—Continued

Scientific and common names	Symptoms resulting from inoculation with—				
	Cabbage virus A	Black ring virus	Cabbage virus B	Cauliflower mosaic virus	Cabbage viruses A and B
B. nigra (L.) Koch (black mustard).	Local: Necrotic spots on inoculated leaves of old plants. Systemic: At 28° vein clearing and chlorosis with rapid necrosis, rosetting, and death. At 20° similar but slightly slower.	Local: Angular necrotic lesions on inoculated leaves; more conspicuous at 20° than 28°. Systemic: At 28° plants becoming chlorotic and dying as if burnt by fire; death in 5 to 6 days after inoculation. At 20° plants stunted, leaves curled inward, growing point becoming necrotic and causing plant to die; much more severe than virus A.	Local: None. Systemic: At 28° vein clearing and mild mottle; severe rosetting, leaf curvature; chlorosis; stunting. At 20° similar to 28° but milder.	Local: None. Systemic: Similar to virus B but much more rapid and severe.	Local: None on young plants. Systemic: Similar to virus A.
Capsella bursa-pastoris (L.) Medic. (shepherd's-purse).	Local: None. Systemic: At 28° vein clearing and diffuse mottle; lower leaves yellow and necrotic; florets malformed; severe stunting; commonly lethal. At 20° similar to 28° and equally severe.	Not tested.	Local: None. Systemic: At 28° vein clearing conspicuous; coarse yellow mottle; mild stunting; general chlorosis.	Local: None. Systemic: Very similar to virus B.	Local: None. Systemic: Similar to virus A but becoming more severe with age.
Matthiola incana var. *annua* (L.) Voss (annual stock, var. Dwarf Large Flowering Ten Weeks).	Local: None. Systemic: At 28° vein clearing and diffuse mottle; leaf malformation; necrosis along and between veins; flowers breaking; stunting. At 20° similar to 28°, but less severe.	Local: None at 28°. At 20° conspicuous necrotic lesions 1 to 2 mm. in diameter, later forming necrotic ring spots. Systemic: At 28° vein clearing and mottling very similar to virus A; considerable leaf distortion. At 20° vein clearing, distortion and mottling, more severe than at 28°; also more severe than virus A at 20°; vein necrosis and leaf distortion marked.	Local: None. Systemic: At 28° vein clearing, mild mottle, occasional chlorotic spots, slight stunting. At 20° vein clearing and chlorotic spots more pronounced; very mild stunting; no flower breaking.	Local: None. Systemic: Similar to virus B but slightly more pronounced.	Local: None. Systemic: At 28° similar to virus A but more severe stunting, malformation, necrosis, and defoliation. At 20° milder than at 28° but more severe than virus A at 20°.

Raphanus sativus L. (radish, vars. Scarlet Globe, Crimson Giant, and French Breakfast).	Local: None. Systemic: At 28° vein clearing, disappearing rapidly; mild mottle; moderate stunting; hypocotyl enlargement retarded; some tan necrotic flecks. At 20° vein clearing with fine mottle; moderate stunting.	Local: None. Systemic: Similar to virus A but milder with only a mild indistinct mottle.	Local: None. Systemic: At 28° conspicuous vein clearing, fine mottle, mild stunting and malformation. At 20° vein clearing and mild mottle, both slightly more prominent than at 28°.	Local: None. Systemic: Similar to virus B but rapidly becoming more pronounced and severe than virus B; some necrosis.	Local: None. Systemic: At 28° similar to virus A with vein clearing becoming more prominent as with virus B. At 20° same except for bright red necrotic flecks with yellow halos.
Lepidium sativum L. (cress, var. Extra Curled).	Local: None. Systemic: At 28° rapid severe stunting and yellowing followed by necrosis and death. At 20° similar to virus A but slightly slower.	Local: None. Systemic: Exactly as for virus A.	Local: None. Systemic: At 28° and 20° vein clearing and diffuse mottle; moderate stunting.	Local: None. Systemic: Indistinguishable from virus B.	Local: None. Systemic: Same as for virus A.
L. virginicum L. (wild peppergrass).	Local: None. Systemic: At 28° vein clearing and mottle; small necrotic spots; entire plant turning purple, wilting, and dying. At 20° similar but milder.	Local: Circular necrotic lesions, more conspicuous at 20° than 28°. Systemic: At 28° chlorotic vein clearing followed by distorting mottle; plants severely stunted and chlorotic; less severe than virus A. At 20° similar to 28° but vein clearing and mottling more conspicuous; more severe than at 28°.	Local: None. Systemic: At 28° and 20° vein clearing becoming prominent; diffuse mottle; golden chlorotic lesions on old leaves.	Local: None. Systemic: Indistinguishable from virus B.	Local: None. Systemic: Same as for virus A.
Thlaspi arvense L. (pennycress).	Local: Necrotic lesions on inoculated leaves at 28°. Systemic: At 28° vein clearing and general chlorosis; marginal necrosis of young leaves; brown discoloration of veins and veinlets; lethal. At 20° similar but slightly slower.	Local: As for virus A. Systemic: At 28° and 20° chlorotic vein clearing, severe golden-yellow chlorosis; severe stunting followed by death; distinctly more severe than virus A. Slightly milder at 20° than at 28°.	Local: None. Systemic: At 28° vein clearing; fine mottle turning to golden yellow; lethal. At 20° similar but more pronounced vein clearing and early necrosis; partly lethal.	Local: None. Systemic: Indistinguishable from virus B.	Exactly similar to virus A.
Neslia paniculata (L.) Desv. (ballmustard).	Local: None. Systemic: At 28° and 20° vein clearing and general chlorosis, some lower leaves rapidly turning yellow and abscising, others becoming necrotic; highly lethal.	Local: None at 28°; at 20° tan necrotic lesions. Systemic: At 28° plants show conspicuous chlorotic vein clearing, yellow mottle, and leaf curvature; death within 10 days after inoculation; more severe than for virus A. At 20° similar to 28° but less severe.	Local: None. Systemic: At 28° and 20° vein clearing, followed by mild mottle.	Local: None. Systemic: Similar to virus B.	Local: None. Systemic: Indistinguishable from virus A.

TABLE 3.—*Symptoms produced on young plants of various species of Cruciferae in the greenhouse at constant temperatures of 20° and 28° C. when inoculated mechanically with cabbage virus A, cabbage black ring virus, cabbage virus B, cauliflower mosaic virus, and cabbage viruses A and B together*—Continued

Scientific and common names	Symptoms resulting from inoculation with—				
	Cabbage virus A	Black ring virus	Cabbage virus B	Cauliflower mosaic virus	Cabbage viruses A and B
Sisymbrium altissimum L. (tumblemustard).	Local: None. Systemic: At 28° mild mottle, stunting, and unilateral development of leaves; necrosis on lower leaves; symptoms quite mild. At 20° plants stunted and rosetted; leaves mottled; old leaves necrotic.	Not tested.	Local: None. Systemic: At 28° and 20° mild yellow mottle and vein clearing; midrib curvature in young leaves.	Local: None. Systemic: Very similar to virus B.	Local: None. Systemic: Similar to virus A.
Brassica juncea (L.) Cosson (Indian mustard).	Local: None. Systemic: At 28° vein clearing; mild mottle becoming chlorotic and necrotic; highly lethal. At 20° similar to 28°; highly lethal.	Local: As described for black mustard. Systemic: As described for black mustard.	Local: None. Systemic: At 28° vein clearing followed by mild mottle, narrowing and wrinkling of leaves. At 20° similar to 28° but finally more severe.	Local: None. Systemic: Similar to virus B but much more severe.	Local: None. Systemic: Similar to virus A.
B. kaber var. *pinnatifidum* (Stokes) L. C. Wheeler (charlock).	Local: None at 28°. At 20° a few angular necrotic lesions. Systemic: At 28° leaves curling and turning dark green; rapid necrosis and death. At 20° similar to 28° but slightly slower.	Local: As described for black mustard. Systemic: As described for black mustard.	Local: None. Systemic: Vein clearing; chlorotic streaks along veins; midrib curved; leaves distorted. At 20° vein clearing and mild mottle, becoming more severe with age.	Local: None. Systemic: Similar to virus B but more rapid and more severe, developing into severe chlorosis and rosette.	Local: None. Systemic: Similar to virus A.
B. napus L. (rape, var. Dwarf Essex).	Local: At 28° occasional zonate brown necrotic spots on inoculated leaves, necrosis spreading along veins, petiole, and stem; becoming lethal. Systemic: At 28° vein clearing and severe mottle and malformation, including reduction of leaf lamina to "shoestring" and formation of blisterlike pockets; severe chlorosis. At 20° similar to 28° but less severe.	Local: None. Systemic: At 28° similar to virus A but less distortion and diffuse chlorosis; no necrosis. At 20° mild fine-type mottle; much milder than for 28°; necrosis on lower leaves more severe than with virus A; lower leaves distinctly chlorotic.	Local: None. Systemic: At 28° vein clearing; prominent, diffuse mottle; midrib curved. At 20° incubation period longer, symptoms more pronounced; stunting mild.	Local: None. Systemic: Similar to virus B but more rapid in development and markedly more severe. At 28° considerable chlorosis and leaf ruffling. At 20° marked rosetting of young growth.	Local: Same as virus A. Systemic: At 28° similar to virus A for about 3 weeks when vein clearing similar to virus B develops; severe stunting. At 20° similar to 28° but slightly less severe.

Species					
B. pekinensis (Lour.) Rupr. var. (Chinese cabbage, Chihli).	Local: None. Systemic: At 28° persistent vein clearing; rapidly developing chlorotic mottle; severe malformation and stunting; tan necrotic spots coalescing or purplish streak of veins extending to petiole and stem; often lethal. At 20° severe diffuse mottle; stunting and distortion.	Local: None. Systemic: Very similar to virus A except stunting, necrosis, and leaf malformation more severe.	Local: Occasional chlorotic lesions produced on inoculated leaves, more so at 20° than at 28°. Systemic: Marked vein clearing; conspicuous chlorotic lesions; necrosis along margins of young leaves; marked curvature of young leaves; rosetting. At 20° similar to 28° but milder.	Local: Same as for virus B. Systemic: Similar to virus B but much more severe. Rosetting more severe at 20° than at 28°.	Local: None. Systemic: At 28° similar to virus A; tan necrosis more severe, mottle more diffuse. At 20° vein clearing more persistent than with virus A.
Berteroa incana (L.) DC. (hoary alyssum).	Local: None. Systemic: At 28° mottle consisting mostly of yellow with green along veins and veinlets; vein clearing; leaf malformation; florets blighted. At 20° diffuse mottle, malformation; slight stunting.	Local: None. Systemic: Mottle and vein clearing very similar to virus A at 28° and 20°.	None.	None.	Local: None. Systemic: Similar to virus A.
Barbarea vulgaris R. Br. (yellow rocket).	None.	None.	do.	do.	None.
Hesperis matronalis L. (dames violet).	Local: None. Systemic: At 28° very conspicuous yellow mottle and vein clearing; leaves severely distorted and stunted; slight vein necrosis. At 20° symptoms similar to 28° but milder.	Local: None. Systemic: Similar to virus A but mottle less conspicuous, less distortion and stunting.	do.	do.	Local: None. Systemic: Same as for virus A.
Iberis amara L. (candytuft).	Local: None. Systemic: Mild vein clearing; mottling; downward curling of young leaves; stunting.	Local: None. Systemic: Indistinguishable from virus A.	Not tested.	Not tested.	Not tested.
Alyssum maritimum Lam. (sweet alyssum).	None.	None.	do.	do.	Do.
Malcomia maritima (L.) R. Br. (Virginian stock).	Local: Circular, tan, necrotic lesions 1 to mm. in diameter. Systemic: Mild mottle, chlorosis; new growth markedly rosetted due to stunting and stimulated growth of adventitious buds; not lethal at 20°.	Local: As for virus A but more pronounced. Systemic: Similar to virus A but decidedly more severe; lethal at 20°.	None.	None.	Do.

TABLE 3.—*Symptoms produced on young plants of various species of Cruciferae in the greenhouse at constant temperatures of 20° and 28° C. when inoculated mechanically with cabbage virus A, cabbage black ring virus, cabbage virus B, cauliflower mosaic virus, and cabbage viruses A and B together*—Continued

Scientific and common names	Symptoms resulting from inoculation with—				
	Cabbage virus A	Black ring virus	Cabbage virus B	Cauliflower mosaic virus	Cabbage viruses A and B
Matthiola bicornis (Sibth. and Sm.) DC. (evening scented stock).	Local: Numerous circular chlorotic lesions 1 to 2 mm. in diameter. Systemic: Vein clearing, mottling, leaf curvature and stunting; plants bleached, becoming necrotic and dying 20 to 25 days after inoculation at 20°.	Local: Conspicuous necrotic lesions 2 to 3 mm. in diameter. Systemic: As for virus A but more severe; plants died 12 days after inoculation at 20°.	Not tested	Not tested	Do.
Cheiranthus cheiri L. (wallflower).	Local: None. Systemic: Chlorotic mottle accompanied by severe leaf and stem curvature; young severely stunted leaves curved unilaterally and downward, forming a marked rosette; more pronounced at 28° than at 20°.	Local: None. Systemic: As for virus A except slightly more severe.	do	do	Do.

TABLE 4.—*Symptoms produced on young noncruciferous plants in the greenhouse at constant temperatures of 20° or 28° C. when inoculated mechanically with cabbage virus A and the cabbage black ring virus*

Scientific and common names	Symptoms resulting from inoculation with—	
	Cabbage virus A	Black ring virus
Nicotiana tabacum L. (tobacco, var. Connecticut Havana No. 38).	Local: Necrotic lesions appearing as small flecks but rapidly expanding to 5 to 8 mm. in diameter; brick-red centers and brown, concentric outlying rings; proximal lesions coalescing to form large necrotic area but still maintaining their individuality; appearing in 3 to 4 days at 28° C. and in 7 to 8 days at 20°. Systemic: None	Local: As for virus A. Systemic: None.
N. glutinosa L	Local: At 28° conspicuous pattern of chlorotic rings and ring spots; no necrosis. At 20° large chlorotic lesions becoming necrotic. Systemic: At 28° zonate chlorotic rings and ring spots; no mottle; no leaf distortion. At 20° numerous chlorotic spots and rings producing a distinct mottle developing into a severe systemic necrosis as the chlorotic lesions become necrotic; considerable stunting and distortion of young leaves.	Local: As for virus A except more rapid in development. Systemic: As for virus A except more severe stunting and less severe necrosis at 20° C.
N. multivalvis Pursh	Local: None Systemic: Chlorotic mottle with considerable distortion of leaf laminae; mild stunting; no necrosis at 28° or 20°.	Local: At 20° small whitish rings about pencil point in size. Systemic: Conspicuous pattern of white rings or pattern of irregular etchings in addition to mottle characteristic of virus A; large, yellow, ring spot lesions on older leaves; stunting and necrosis severe, many plants killed; much more severe than virus A; more severe at 20° than at 28°.
N. paniculata L	Local: At 28° lesions ranging from tan spots to tan rings 1 to 2 mm. in diameter, expanding and developing into pattern of large concentric rings and ring spots; no necrosis. Systemic: None	Local: As for virus A except for tendency of concentric rings to develop necrotic centers. Systemic: None.
N. quadrivalvis Pursh	Local: None Systemic: At 28° conspicuous mottle with considerable stunting and distortion of leaves; no necrosis; no etching pattern as with black ring virus.	Local: None. Systemic: Mottle as for virus A; white etching pattern as in *N. multivalvis* but less pronounced.
N. rustica L	Local: None at 28°. Inconspicuous chlorotic lesions, occasionally necrotic at 20°. Systemic: At 28° chlorotic rings and solid, irregular chlorotic spots; some rings zonate; with age ring development ceasing and only chlorotic spots developing; some leaf distortion. At 20° only irregular chlorotic lesions developing, giving a coarse mottle effect.	Local: Few chlorotic lesions at 28°. Conspicuous chlorotic rings and necrotic lesions at 20°. Systemic: Much like virus A except zonate rings more prevalent than in virus A. At 20° numerous chlorotic rings and ring spots tending to become necrotic; decidedly more conspicuous than at the higher temperature.
N. acuminata (Graham) Hook	Local: At 28° tan chlorotic lesions tending to become necrotic. Systemic: Chlorotic lesions, later becoming necrotic; young leaves severely puckered and drawn.	Local: At 28° tan necrotic lesions with a dark-brown periphery and surrounded by a chlorotic halo. Systemic: As for virus A except more necrotic.
N. alata var. *grandiflora* Comes	Local: At 28° whitish rings and ring spots tending to become necrotic. Systemic: None	Local: At 28° necrotic lesions surrounded by white rings. Systemic: None

Table 4.—*Symptoms produced on young noncruciferous plants in the greenhouse at constant temperatures of 20° or 28° C. when inoculated mechanically with cabbage virus A and the cabbage black ring virus*—Continued

Scientific and common names	Symptoms resulting from inoculation with—	
	Cabbage virus A	Black ring virus
✕ *N. sanderae* W. Wats	Local: At 20° circular tan chlorotic lesions tending to consist of rings; developing large, diffuse, chlorotic areas with age; no necrosis. Systemic: None.	Local: At 20° as for virus A except for tendency of lesions to develop necrotic borders. Systemic: None.
N. sylvestris Speg	Local: At 28° necrotic lesions very similar to those on *N. tabacum*, but developing 8 to 10 days later. Systemic: None.	Local: At 28° as for virus A but slightly more rapid in development. Systemic: None.
N. trigonophylla Dunal	Local: At 20° numerous chlorotic rings and lesions. Systemic: Large chlorotic lesions tending to consist of 6 to 7 concentric chlorotic rings; no necrosis; no distortion.	Local: At 20° as for virus A. Systemic: Similar to virus A but lesions largely spots with bright-yellow centers and little or no ringing.
N. longiflora Cav	Local: At 28° whitish rings 1 to 2 mm. in diameter. Large golden-yellow spots on old plants. Systemic: None.	Local: At 28° as for virus A except rings 2 to 4 mm. in diameter; large yellow-green spots on old plants. Systemic: None.
N. nudicaulis S. Wats	Local: At 28° none. Systemic: Very mild mottle with lesions, tending to become necrotic; symptoms very mild.	Local: At 28° none. Systemic: Only slight chlorosis along the veins; virus recovered in high concentration.
Physalis pubescens L. (husk tomato).	Local: At 28° circular chlorotic lesions. Systemic: Numerous chlorotic lesions and vein clearing resulting in a true mottle effect; young leaves severely stunted and distorted; severe defoliation, leaving only a tuft of stunted leaves at the growing point.	Local: As for virus A. Systemic: Exactly as for virus A except for conspicuous chlorotic ringing and vein banding on some plants.
Solanum integrifolium Poir. (Chinese scarlet eggplant).	Local: Black, irregular to circular, necrotic lesions and streaking of the veins at both 28° and 20°. Systemic: At 28° and 20° much as on inoculated leaves; no mottle; no vein clearing; only systemic necrotic lesions and vein streaking with some puckering of leaf midrib; severe stunting.	Local: None. Systemic: No infection in 4 trials.
S. rostratum Dunal (buffalo-bur).	Local: At 28° few faintly necrotic lesions remaining inconspicuous. Systemic: None.	Local: Conspicuous small, brown, necrotic lesions at 28°. Systemic: None.
S. pseudo-capsicum L. (Jerusalem-cherry).	None.	None.
S. dulcamara L. (bittersweet)	____do____	Do.
Datura metel L	Local: At 28° broad, irregular chlorotic areas along and between the veins; interveinal chlorotic lesions. Systemic: Numerous circular, chlorotic lesions on the leaves with slight puckering of the leaf laminae.	Local: As for virus A. Systemic: Exactly as for virus A.
D. meteloides Dunal	Local: At 28° circular chlorotic lesions. Systemic: Conspicuous mottle with mildly stunted leaves.	Local: As for virus A. Systemic: Exactly as for virus A.
D. stramonium L. (jimsonweed)	None.	None.
Atropa belladonna L. (belladonna).	____do____	Do.
Capsicum frutescens var. *cerasiforme* (Mill.) Bailey (cherry pepper).	____do____	Do.

TABLE 4.—*Symptoms produced on young noncruciferous plants in the greenhouse at constant temperatures of 20° or 28° C. when inoculated mechanically with cabbage virus A and the cabbage black ring virus*—Continued

Scientific and common names	Symptoms resulting from inoculation with—	
	Cabbage virus A	Black ring virus
Lycium halimifolium Mill. (matrimony-vine).	Local: At 28° tan chlorotic leisons never becoming necrotic. Systemic: None.	Local: Tan necrotic lesions. Systemic: None.
Nicandra physalodes (L.) Pers. (apple-of-Peru).	Local: At 28° few small, chocolate-brown lesions increasing in size, becoming irregular in outline and finally surrounded by chlorotic halos. Systemic: None.	Local: As for virus A except much more numerous and developing more rapidly. Systemic: None.
Salpiglossis sinuata Ruiz and Pav.	Local: None⸺⸺⸺⸺ Systemic: At 28° very mild indistinct mottle; conspicuous vein clearing; mild stunting; no necrosis.	Local: None. Systemic: Mild but distinct fine-type mottle; no conspicuous vein clearing; moderate stunting; no necrosis.
Beta vulgaris var. *cicla* L. (Swiss chard, var. Lucullus).	Local: Tan to red chlorotic flecks, occasionally necrotic; slightly more pronounced at 28° than at 20°. Systemic: At 28° brick-red to tan spots occasionally surrounded by yellow halos; slight stunting; at 20° similar to 28°.	Local: Severe brick-red necrotic flecking; distinctly more necrotic than virus A. Systemic: As for virus A but necrosis more severe than virus A at both 28° and 20°.
B. vulgaris L. (sugar beet, var. U. S. 200 × 215).	Local: At 28° tan to red chlorotic to necrotic flecks. Systemic: Small, circular or irregular, brown to red necrotic lesions up to 2 mm. in diameter, sometimes coalescing; occasionally a faint mottle.	Local: Red necrotic flecking more severe than virus A. Systemic: As for virus A but more severe.
Spinacia oleracea L. (spinach, vars. Old Dominion, Giant Thick Leaf, Bloomsdale).	Local: None⸺⸺⸺⸺ Systemic: At 28° vein clearing, chlorotic mottle; young leaves rapidly becoming chlorotic; stunted, puckered; growing points becoming a rosette of "shoestring" leaves; severe stunting; lower leaves becoming necrotic and abscising; stunted rosette dying in 3 to 4 weeks. At 20° slower, diffuse mottle, and vein clearing; moderate stunting and leaf distortion.	Not tested.
Amaranthus tricolor L. (pigweed).	Local: Small but conspicuous necrotic lesions at 28° and 20°. Systemic: Conspicuous, chlorotic vein clearing and diffuse mottle; stunting and premature leaf abscission.	Local: As for virus A. Systemic: As for virus A except no mottle.
Celosia cristata L. (cockscomb).	Local: At 20° tan necrotic lesions 2-4 mm. in diameter. Systemic: Slight stunting and leaf distortion; very mild mottle.	Local: As for virus A except appearing 4–5 days earlier. Systemic: Exactly as for virus A.
Chenopodium album L. (lambsquarters).	Local: At 28° tan, circular necrotic lesions 1-2 mm. in diameter, tan centers with dark-brown borders somewhat zonate. Systemic: Small chlorotic lesions becoming necrotic; stunting; distortion.	Local: As for virus A. Systemic: As for virus A but slightly more severe.
Dolichos lablab L. (hyacinth-bean)	None⸺⸺⸺⸺	None.
Phaseolus coccineus L. (scarlet runner bean).	⸺do⸺⸺	Do.
Soja max (L.) Piper (soybean)	⸺do⸺⸺	Do.
Vigna sinensis (Torner) Hassk (cowpea, var. Blackeye).	⸺do⸺⸺	Do.

TABLE 4.—*Symptoms produced on young noncruciferous plants in the greenhouse at constant temperatures of 20° or 28° C. when inoculated mechanically with cabbage virus A and the cabbage black ring virus*— Continued

Scientific and common names	Symptoms resulting from inoculation with—	
	Cabbage virus A	Black ring virus
Ipomoea purpurea (L.) Roth. (morning-glory).	None_____ ____do_____	None. Do.
Digitalis purpurea L. (foxglove).	Local: None_____ Systemic: At 28° inconspicuous chlorosis along the veins; few scattered chlorotic lesions; symptoms very mild.	Local: None. Systemic: Chlorosis along veins; mottle very conspicuous in some plants, much more so than virus A.
Hibiscus esculentus L. (okra, var. Long Velvet).	None_____	__Not tested.
Abutilon theophrasti Medic. (velvet leaf).	Local: None_____ Systemic: At 20° mild chlorotic mottle becoming masked with age; slight stunting but no necrosis or leaf malformation.	Local: None. Systemic: As for virus A except mottle less conspicuous; virus not removed in inoculation to tobacco.
Mirabilis jalapa L. (four-o'clock).	None_____	None.
Verbena hybrida Voss (garden verbena).	Local: At 20° necrotic lesions 5 to 6 mm. in diameter, with tan centers and chocolate-brown peripheries. Systemic: Mild mottle, chlorosis and slight leaf distortion.	Local: At 20° as for virus A except lesions more numerous. Systemic: Mild mottle, chlorosis, leaf distortion, and vein necrosis.
Centaurea nigra L. (knapweed).	None_____	None.
Tagetes patula L. (French marigold).	____do_____	Do.
Vinca minor L. (periwinkle).	Local: None_____ Systemic: At 28° circular chlorotic lesions with tan borders producing a ring effect; no stunting or distortion.	Local: None. Systemic: As for virus A except rings more numerous and more conspicuous.
Stellaria media (L.) Cyrill. (chickweed.	Local: None_____ Systemic: At 20° mild mottle and chlorotic vein banding; no stunting; no necrosis.	Local: None. Systemic: As for virus A except mottle more conspicuous and leaves slightly distorted.
Scabiosa atropurpurea L. (mourning bride).	Local: None_____ Systemic: At 20° chlorotic mottle with vein clearing; leaves stunted and distorted; no necrosis.	Local: None. Systemic: As for virus A except much more severe; mottle and necrosis severe.
Godetia amoena Lilja (farewell-to-spring).	Local: None_____ Systemic: At 28° conspicuous chlorotic mottle; stunting and distortion of young leaves; proliferation of adventitious growth in some plants.	Local: None. Systemic: Very similar to virus A.
Delphinium ajacis L. (rocket larkspur).	Local: At 28° crescent-shaped necrotic lesions along leaf margin. Systemic: Chlorosis, yellow mottle, necrosis as on inoculated leaves; necrosis of stem and floral buds; plants severely stunted and with a scorched appearance; flower breaking in form of white streaks in the corolla.	Local: As for virus A but developing 7 to 10 days earlier. Systemic: As for virus A but 7 to 10 days earlier; more pronounced than virus A in early stages but ultimately quite similar.
Reseda odorata L. (mignonette).	Local: At 28° chlorotic lesions occasionally necrotic. Systemic: Conspicuous vein clearing and mild mottle; marked stunting and distortion of young leaves; severe necrosis of veins causing entire leaves to die on some plants.	Local: Necrotic lesions, only occasionally chlorotic. Systemic: As for virus A except necrosis more severe.
Calendula officinalis L. (calendula, var. Orange King).	Local: None_____ Systemic: At 20° chlorotic mottle; stunting, distortion, and general chlorosis of young leaves; necrotic spotting on some leaves.	Local: None. Systemic: Indistinguishable from virus A.

TABLE 4.—*Symptoms produced on young noncruciferous plants in the greenhouse at constant temperatures of 20° or 28° C. when inoculated mechanically with cabbage virus A and the cabbage black ring virus*—Continued

Scientific and common names	Symptoms resulting from inoculations with—	
	Cabbage virus A	Black ring virus
Centaurea moschata L. (sweet-sultan).	Local: None. Systemic: At 20° mottle with vein clearing; severe stunting and distortion; not lethal.	Local: None. Systemic: As for virus A except development much more rapid and severe; lethal at 20° within 3 weeks after inoculation.
Dimorphotheca aurantiaca DC. (winter Cape-marigold).	Local: None. Systemic: At 28° chlorotic vein clearing and mottle; marked distortion of young leaves; rapidly lethal.	Local: None. Systemic: Exactly as for virus A.
Helianthus annuus L. (sunflower, var. Giant Russian Mammoth).	Local: Black necrotic lesions 3 to 4 mm. in diameter with tan centers and black peripheries at both 28° and 20°. Systemic: Numerous chlorotic lesions becoming necrotic; necrosis finally involving entire leaf causing it to abscise; symptoms alike at 20° and 28°.	Local: As for virus A. Systemic: Exactly as for virus A.
Senecio cruentus DC. (florist's cineraria).	Local: Tan necrotic rings at 28° and 20°. Systemic: Mild vein clearing and chlorosis of young leaves; stunting and distortion very mild; milder at 20° than at 28°.	Local: Irregular necrotic pattern but not rings as in virus A. Systemic: Conspicuous vein clearing; mild mottle; vein necrosis; considerable distortion. More severe at 28° and 20° than virus A.

SYMPTOMS ON CRUCIFEROUS HOSTS

The symptoms of the various viruses on the different cruciferous hosts are given in table 3. It may be well to point out here some of
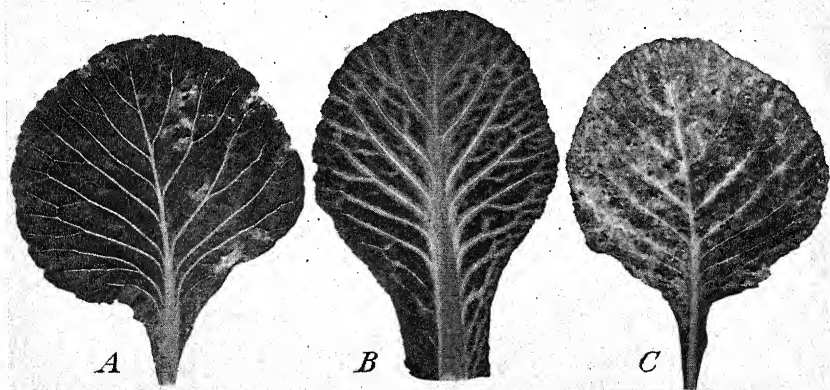
FIGURE 1.—Leaves from cabbage plants systemically infected: *A*, Cabbage virus A at 28° C.; *B*, cabbage virus B at 16°; *C*, cabbage viruses A and B at 28°. Photographed 5 weeks after inoculation. Note the mottle effect of virus A at 28° and the intensification of mottle at this temperature when virus B is present. Note also the vein clearing caused by virus B at 16°.

the cardinal points of similarity and contrast. Cabbage virus B had a longer incubation period by several days than cabbage virus A. The latter usually first caused a mottle at 28° C. (fig. 1, *B*) and,

although vein clearing was often evident, it tended to be obscured rather promptly as the mottle became more pronounced. Necrosis was not common with virus A on cabbage but appeared as purple rings on cauliflower and streak necrosis on brussels sprouts; leaf malformation was common. On wild mustards, pennycress, and peppergrass the disease caused by virus A progressed rapidly and was usually lethal. At 20° this virus was generally distinctly milder than the black ring virus in rate of progress and severity of symptoms, although necrosis and premature defoliation were sometimes more pronounced on certain hosts than at 28°.

Cabbage virus B usually caused vein clearing and a mild diffuse mottle at 28° C. Vein clearing increased in prominence, but
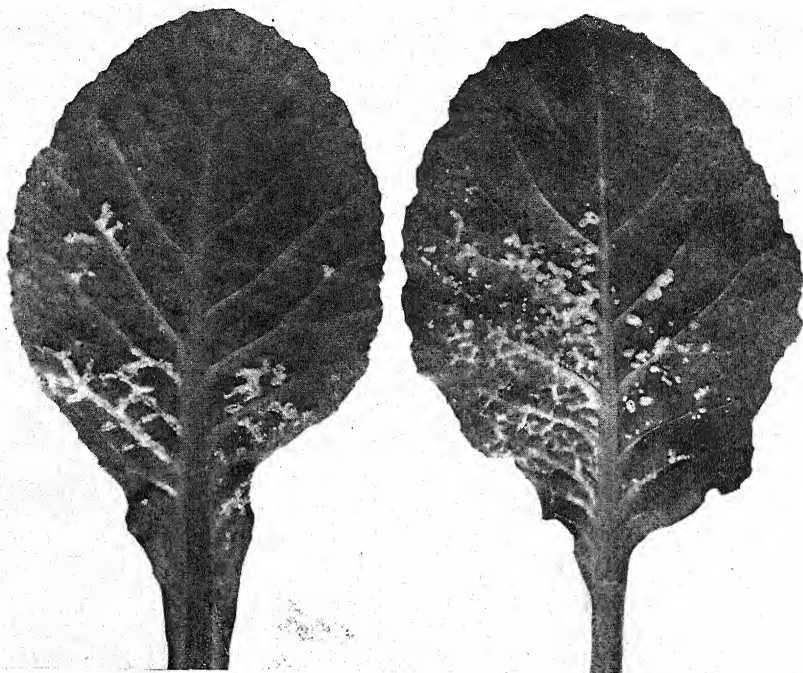
FIGURE 2.—Enations on cabbage leaves systemically infected with cabbage virus B. Photographed 60 days after inoculation and incubation at 12° C.

eventually all symptoms other than stunting and chlorosis became masked at this temperature. At 20° vein clearing appeared more slowly than at 28°, but it gradually increased until it became the prominent permanent characteristic. At 16° vein clearing was still more pronounced than at 20° (fig. 1, *B*). At low temperatures enations on systemically infected leaves of cabbage were common (fig. 2). It may not be amiss, therefore, to refer to virus B as the vein clearing virus and to virus A as the mottle virus.

When viruses A and B were inoculated together the symptoms were very similar to those described earlier for cabbage mosaic,[13] and they were more truly representative of the disease as it occurred in nature

[13] See footnote 3.

in southeastern Wisconsin. When plants were kept at 28° C. the symptoms were most like those of virus A. As a rule, however, mottle was more severe and necrosis more common and destructive (fig. 1, *C*). It is evident that at the high temperature virus A predominated, but the symptoms were more severe than when only virus A was present. At 20° the course of the disease was retarded as compared with 28° and the symptoms of virus B gradually became predominant. In such plants as white mustard, black mustard, and charlock, in which the action of virus A was rapid and lethal at 28° and nearly as severe at 20°, there was little or no evidence of any influence of the slowly acting virus B on the course of the disease.

The symptoms produced by cabbage virus B and the cauliflower mosaic virus were in general very similar, giving further evidence
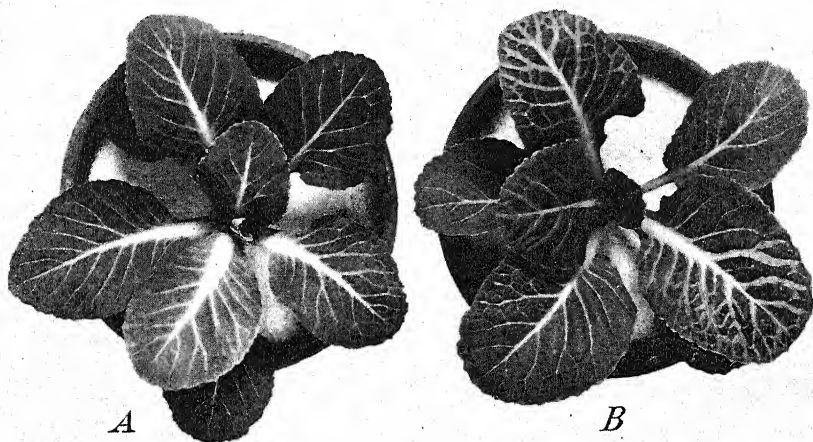
FIGURE 3.—Cabbage plants showing effect of the cauliflower mosaic virus (*A*) and of the cabbage virus B (*B*), 42 days after inoculation at 12° C. Note the more prominent vein clearing with cabbage virus B.

that they are closely related. Certain differences, however, are worthy of note. On cabbage the cauliflower mosaic virus was distinctly milder in its effect (fig. 3), while on cauliflower the reverse was true. On broccoli the cauliflower mosaic virus was definitely milder at first but gradually became indistinguishable from virus B. On turnip the cauliflower mosaic virus was the more severe early, but gradually the two became indistinguishable. On kale, brussels sprouts, cress, peppergrass, pennycress, ballmustard, and tumblemustard the two were nearly identical. On yellow mustard, white mustard, black mustard, Indian mustard, charlock, rape, Chinese cabbage, and radish the cauliflower mosaic virus caused more rapid disease development and the symptoms were much more severe (fig. 4).

The black ring virus produced symptoms rather similar to those of cabbage virus A. There were some marked differences, however, which are worthy of note. On cabbage the black ring virus was less pronounced at 28° C. but more severe at 20°, while with cabbage virus A the reverse was true. At the latter temperature black spots
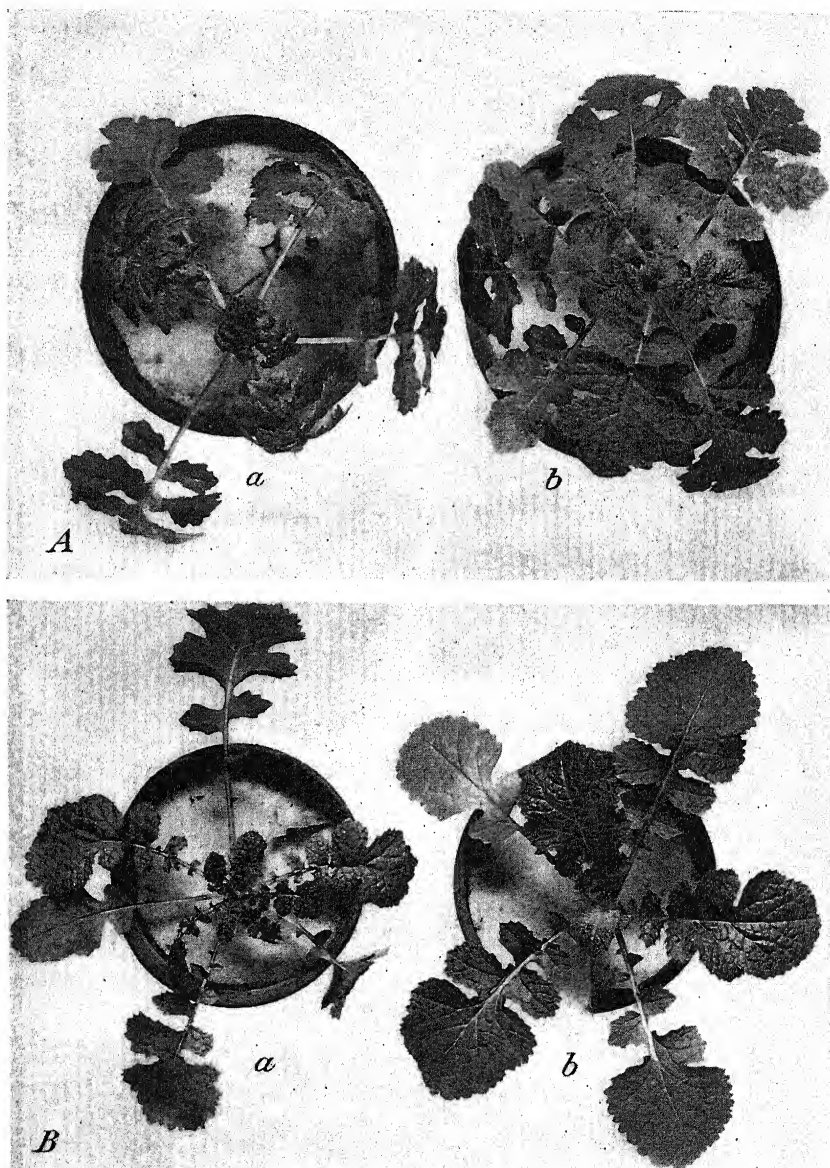
FIGURE 4.—Wild mustards showing effects of the cauliflower mosaic virus (*a*)
and of the cabbage virus B (*b*), 21 days after inoculation. *A*, White mustard
plants at 28° C. Note extreme rosetting caused by the cauliflower virus.
B, Indian mustard plants at 20°. The symptoms of virus B on this host
become more pronounced after longer incubation but never reach the severity
of those associated with the cauliflower virus.

and black rings were characteristic of the black ring virus, while chlorotic spots with relatively mild necrosis were the more common with virus A. This relative difference also prevailed on cauliflower.

SYMPTOMS ON NONCRUCIFEROUS HOSTS

As already pointed out, cabbage virus B and cauliflower mosaic virus caused no symptoms on noncruciferous plants. Cabbage virus A and black ring virus had identical host ranges as far as tested, except that the latter did not affect Chinese scarlet eggplant. The black ring virus had an incubation period shorter by 1 to 7 days on some hosts. Symp-
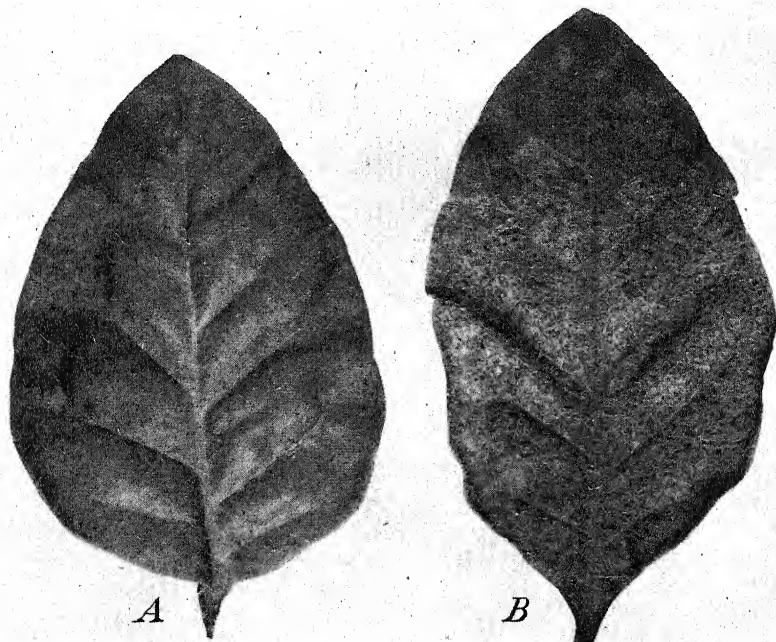
FIGURE 5.—Systemically infected leaves of *Nicotiana multivalvis* showing effects of cabbage virus A (*A*) and of the black ring virus (*B*). Note the very mild chlorotic mottle in *A* caused by cabbage virus A and the marked etching pattern in *B* caused by the black ring virus.

toms of the two viruses (table 4) could usually be distinguished but in most cases only in minor respects. The progression of symptoms was usually more rapid with black ring virus in that necrosis appeared first and the disease reached its most severe stage first. On some hosts the appearance of chlorotic rings and ring spots and the transition of the rings into necrotic areas were pronounced with the black ring virus and relatively inconspicuous with virus A. As a rule, under the conditions of the greenhouse tests, black ring infection resulted in more necrosis and the death of more plants than did infection with virus A.

Certain more distinctive symptoms were to be noted in some hosts. In *Nicotiana multivalvis*, for instance, a conspicuous pattern of white rings was produced by the black ring virus in addition to the type

of chlorotic mottle produced by virus A (fig. 5). A similar distinction was noted with *N. quadrivalvis*. On the other hand, in the case
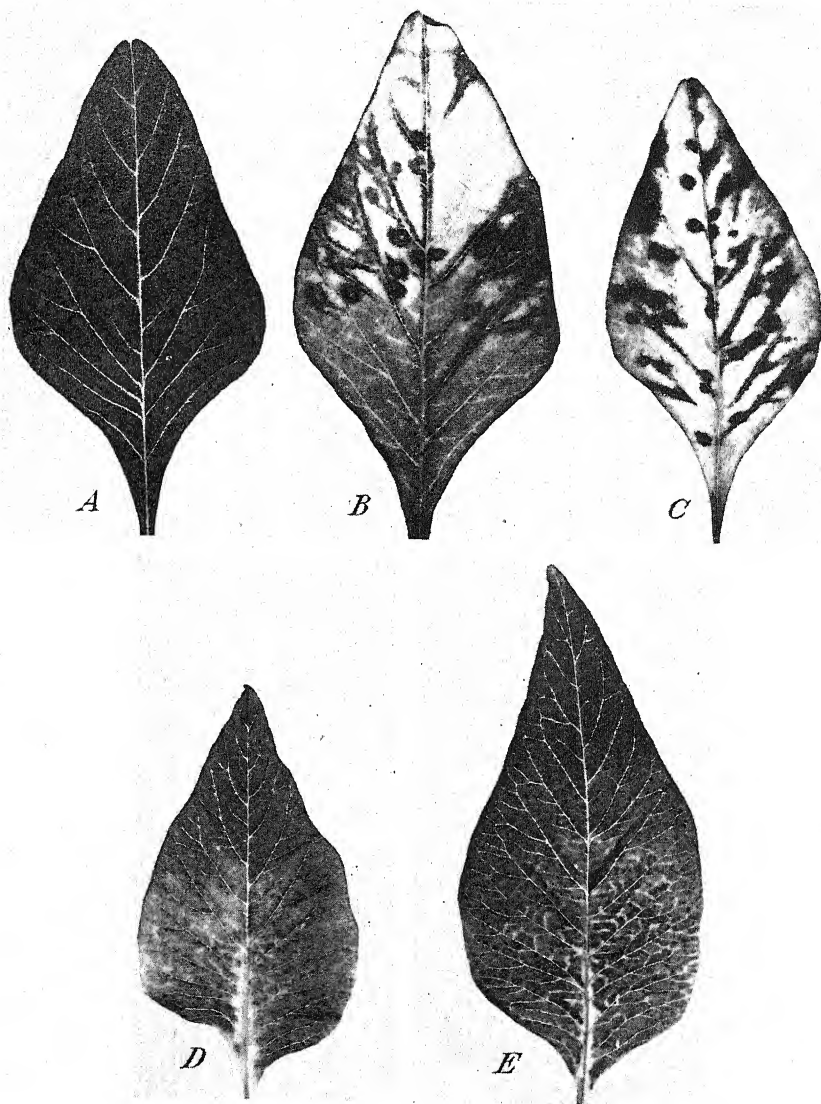
FIGURE 6.— Leaves of *Amaranthus tricolor* showing the effects of cabbage virus A and of the black ring virus. *A*, Uninoculated leaf. *B*, Leaf inoculated with cabbage virus A. *C*, Leaf inoculated with the black ring virus. Note pigmented lesions on a diffuse chlorotic background. *D, E*, Systemically infected leaves from the plants that produced leaves shown in *B* and *C*, respectively. Note diffuse mottle with virus A and chlorotic vein clearing with the black ring virus.

of *Amaranthus tricolor* conspicuous pigmented lesions appeared on inoculated leaves with both viruses, while on systemically infected

leaves there was a diffuse mottle with virus A and a chlorotic vein clearing with the black ring virus (fig. 6).

As is to be expected, the inoculation of each noncruciferous host with a mixture of cabbage viruses A and B led to the same results as inoculation with virus A alone.   This is in contrast to the results obtained with cruciferous hosts susceptible to both viruses in which a combined pathological effect was often distinct from and more severe than that of each virus when inoculated separately.

DISCUSSION

At the present time there is much confusion in the differentiation and classification of plant viruses.   Some of this is due to the inadequate methods of defining them.   While symptoms, host ranges, and such physical properties as those indicated by inactivation of virus extracts by aging in vitro, by heat, and by dilution are helpful, they may lead to overemphasis of relatively minor differences.   The constant danger that more than one virus may be involved in a disease complex adds to the confusion when differentiation by these methods alone is involved.

The results reported here show that two distinct viruses are concerned in cabbage mosaic as found in the Middle West and in western Washington.   One of the components is shown to resemble the black ring virus described in California as to host range, properties, and symptoms, while the other closely resembles the cauliflower mosaic virus described in that State.   It is equally obvious from a study of the disease reaction of many hosts that in the case of each pair the viruses are not identical.   The distinctions between the black ring virus and cabbage virus A on the one hand and between the cauliflower mosaic virus and cabbage virus B on the other are concerned primarily with disease progression and incubation period rather than with physical properties and host range.   Thus, the cauliflower mosaic virus causes more severe disease reaction in cauliflower than in cabbage, while with cabbage virus B the reverse is true.   Many other points of distinction are to be found.   Cabbage virus A causes typically chlorotic and mottle effects, while the black ring virus causes dark-green rather than chlorotic rings and necrotic ring spots.

It seems only logical to conclude, therefore, that cabbage virus A and the black ring virus are closely related strains.   They are, undoubtedly, also closely related to the virus described by Hoggan and Johnson [14] from turnip.   According to the Johnston system of classification, it is the belief of the writers that they should all be classed under turnip virus 1 Hoggan and Johnson.   The ring necrosis virus of Larson and Walker [15] has a very similar host range, although the properties reported are different.   It is believed, therefore, that this is also another strain of the same virus.

The cauliflower mosaic virus is obviously quite distinct from turnip virus 1.   Cabbage virus B is undoubtedly a strain of the cauliflower virus.   The virus from broccoli described from southern England recently by Caldwell and Prentice [16] appears to be closely

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[14] Hoggan, I. A., and Johnson, J. A virus of crucifers and other hosts. Phytopathology 25: 640–644, illus. 1935.
[15] See footnote 6.
[16] Caldwell, J., and Prentice, I. W. A mosaic disease of broccoli. Ann. Appl. Biol. 29: 366–373, illus. 1942.

related. This group of strains is referred to as cauliflower virus 1, and various other strains of it will undoubtedly be found.

It is obvious that when the cabbage A strain of turnip virus 1 and the cabbage B strain of cauliflower virus 1 infect cabbage together the disease differs in many aspects from that which results when either affects this host alone. The combined effect is that which occurs naturally in the Middle West in the disease known as cabbage mosaic.

SUMMARY

Cabbage mosaic as it occurs in the Middle West has been shown to consist of two causal virus entities tentatively designated as cabbage viruses A and B. These two viruses were also recovered from diseased plants in western Washington.

Studies of symptoms, host ranges, and physical properties show that cabbage virus A is very similar to but nevertheless consistently distinct from the black ring virus described from California,[17] while cabbage virus B is very similar to but distinct in some respects from the cauliflower mosaic virus also described from California.[18]

It is suggested that cabbage virus A, the cabbage black ring virus, and the cabbage ring necrosis virus are all strains of turnip virus 1 Hoggan and Johnson. It is further suggested that the cauliflower mosaic virus as described by Tompkins [18] be designated as cauliflower virus 1 and that cabbage virus B and the broccoli virus of Caldwell and Prentice [19] be designated as strains thereof.

[17] See footnote 5.
[18] See footnote 4.
[19] See footnote 16.

RESPONSE OF SHORTLEAF AND PITCH PINES TO SOIL AMENDMENTS AND FERTILIZERS IN NEWLY ESTABLISHED NURSERIES IN THE CENTRAL STATES [1]

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INTRODUCTION

In the early years of production of pine planting stock in the Central States much difficulty was experienced in nurseries newly established on land previously used for agriculture. In many cases only a few small clumps of seedlings survived in each seedbed and height growth of the seedlings was very irregular. The needles on some seedlings were yellow, on others purple, instead of the normal green. Loss from damping-off was excessive before emergence and for a week or two afterward. A thin crust of alkaline soil formed on some surfaces as a result of concentration of calcium salts from capillary and sprinkling water. Most of the soils were low in organic matter. Some were alkaline because of lime natural to the site or distributed during grading operations. Some drainage difficulties were encountered. For these reasons experiments in soil amendment and fertilization were undertaken in three Forest Service nurseries located at Chillicothe, Ohio, Vallonia, Ind., and Licking, Mo. All the nursery areas had previously been in farm crops; consequently there was no information regarding their soil deficiencies for pine seedlings. Cross-sectional research therefore seemed more suitable than isolated studies on specific problems. This paper covers the results of the first 5 years' experimentation.

DESCRIPTION OF NURSERIES

The Chillicothe nursery is situated on a terrace of the preglacial Tuyes River, the topography of which was changed from level to rolling by terminal moranic deposits of the Illinoian glacier. The material of the rolling surface is predominantly sandy in texture, but not homogeneous. Exposure in a cut nearby shows a 12- to 18-inch sandy loam surface horizon with a tight subsoil of sand, gravel, and clay, and sand and gravel below this to a depth of approximately 20 feet. The nursery area itself includes a hill of sandy material without much subsoil development, highly calcareous throughout its profile. Below the zone of moranic influence lies terrace material of a heavier texture. The surface soil is yellowish brown in color, the subsoil predominantly reddish brown but lighter in some spots. In its undisturbed state, this soil probably should be classified as Holston sandy loam.

As the topography was a little too rolling for easy tillage, the surface was leveled. The topsoil was scraped and piled, then replaced after the leveling operation. Unfortunately, more colloidal material was present than might have been expected in a soil of such light

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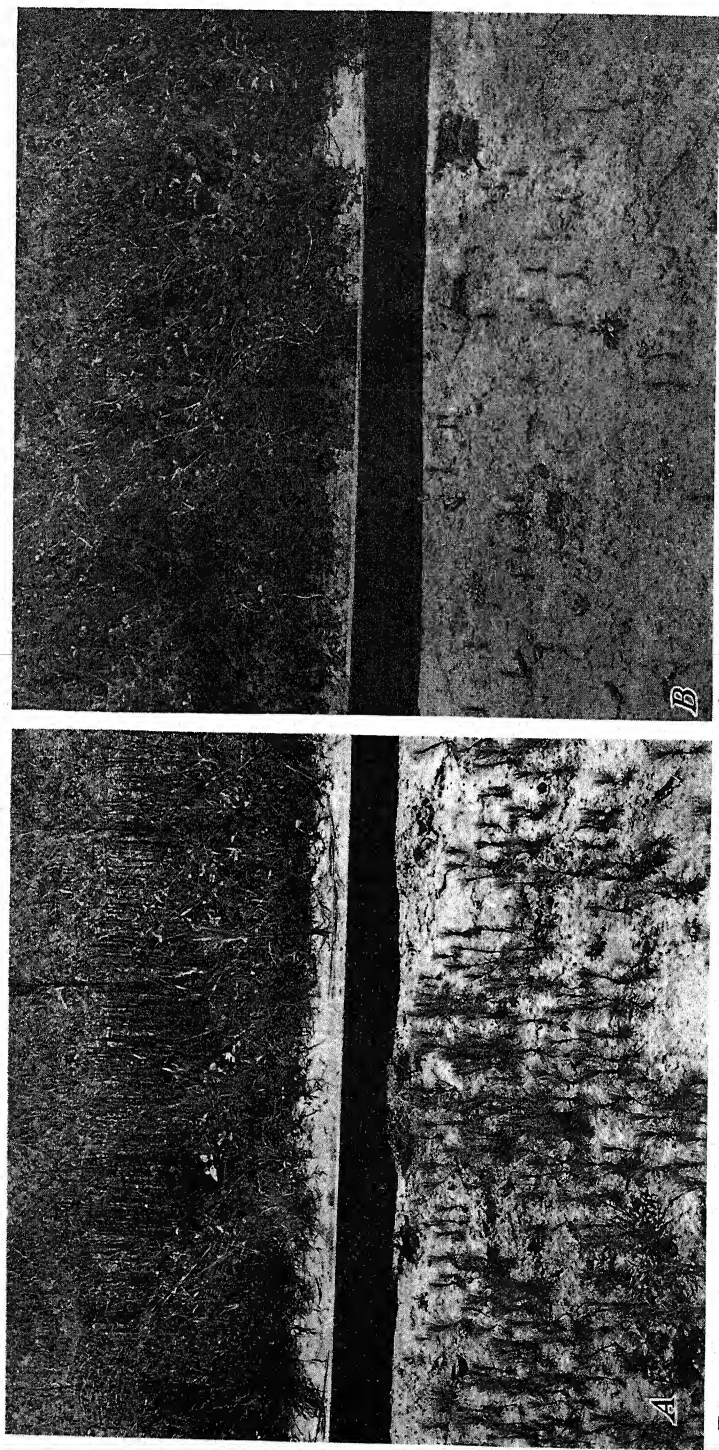
FIGURE 1.—Two views of seedlings in the same seedbed and nearby vegetation in the Chillicothe nursery 1937: *A*, good pitch pine seedlings and poor clover, on acid soil (pH 5); *B*, poor pitch pine seedlings and luxuriant clover, on sweet soil (pH 8)

surface texture, and on many areas scraping exposed the subsoil. Replacing the surface material did not, of course, restore the original normal drainage and aeration. The natural structure had been destroyed by compaction with heavy grading machinery. Grading had exposed calcium carbonate and spread it over a portion of the area. On this portion the surface soil had a reaction above neutrality

FIGURE 2.—Crusting of soil as a result of concentration of calcareous salts in soil water. Chillicothe nursery, 1937

which affected pine seedlings adversely (fig. 1). In general, however, excessive alkalinity was confined to a surface crust not more than a quarter of an inch thick (fig. 2) and the subsurface soil had a reaction of pH 5 to 6. The surface crusting had taken place as a result of concentration of calcium salts from evaporation of soil water.

The Vallonia nursery is located in Jackson County, Ind., on a portion of one of the Shelbyville moraines of the early Wisconsin

glaciation   Its site is composed of high terraces, gently sloping except
for a dunelike hill of wind-blown sand.   The soil has not been surveyed
officially, but would probably be classified as Princeton sandy loam.
The surface material is a yellowish brown sand to sandy loam, becom-
ing more yellow with depth.   At 10 feet some clay streaks occur and
the texture is heavier.   All parts of the area have good drainage.
The soil is deficient in organic matter and in the inorganic colloidal
fraction; hence base-exchange capacity is limited.   At Vallonia, as at
Chillicothe, the surface soil was alkaline, and after rain or watering a
shallow crust frequently developed from concentration of soluble salts.
A considerable part of the incrusting material had been formed by
evaporation of sprinkling water, which came from calcareous material.

The nursery at Licking, Mo., lies chiefly on a terrace of Huntington
silt loam, one corner extending onto a gentle slope of Hanceville fine
sandy loam.   The soil is somewhat heavier than the soils of the Chilli-
cothe and Vallonia nurseries.   Consequently it has an advantage over
them in capacity for retaining soluble fertilizers, owing to higher
colloid content.   Here the sprinkling water was not excessively cal-
careous, the soil was slightly acid, and surface crusting did not take
place.

EXPERIMENTAL PLAN AND PROCEDURE

The exploratory experiments reported here were designed to answer
questions regarding (1) nutrient deficiencies in the soil limiting thrift
of pine seedlings; (2) quantities of the various elements needed; (3)
form of fertilizing material best suited to nursery use; (4) time of
application most favorable; (5) a method of adjusting soil acidity; (6)
effect of incoporating peat into the soil; (7) influence of soil reaction
on fertilizer effects; and (8) effects of inoculation with pine duff.

The plan of experimentation was practically the same in all cases.
Nursery beds 4 feet wide were marked off into as many 3-foot sections
as were necessary for the projected number of tests.   If the number
of plots was large the bed was first divided into blocks.   By allocating
each replicate to a separate block, any possible influence of variation
in soil from one end of the nursery bed to the other was reduced.
Fertilizers were applied in dry form, by working them into the
surface soil before seeding, or in solution after seeding.   Seedling
density counts and height measurements on each plot were taken
on a 1-foot strip crossing the plot at its midpoint.   At digging time
this 1- by 4-foot strip of trees was undermined to prevent breaking
the roots.   The seedlings were allowed to fall into the trench and
were then washed and bundled, and the bundles were labeled with
plot numbers.   In the laboratory, the roots and tops of the seedlings
were measured and weighed.

Averages for single plots were compared with the general average
for all plots in the bed.   To avoid confusing increase in height from
crowding with that directly attributable to fertilizer, a preliminary
test for covariance between height and density was made, and if it
appeared, a correction was introduced.

1936 EXPERIMENT

At the Vallonia nursery the sprinkling water was modified in 1936
by adding sulfuric acid.   This reduced the soil reaction temporarily,
but was discontinued because of the danger of excessive leaching of

bases. Another expedient tried was to work acid-saturated peat into the soil. A core of soil 4 inches deep was mixed with a core of peat of the same diameter 1 inch deep. Sufficient water was added to the soil-peat mixture, in a large beaker, to allow easy stirring, and 1/10 normal sulfuric acid was introduced from a burette. When the reaction, tested by use of a glass electrode, reached pH 5.8 the quantity of acid used was recorded. From this and the weight of peat, the quantities of acid and peat necessary for application in practice were calculated. This treatment remedied the crusting and alkalinity. Peat also acted as a buffer against accumulation of calcium carbonate. This addition, together with less frequent but heavier applications of sprinkling water, solved the calcium problem.

1937 EXPERIMENTS

CHILLICOTHE NURSERY

The first of the series of fertilizer experiments was started at Chillicothe in the spring of 1937 with pitch pine (*Pinus rigida*). Potassium sulfate, ammonium nitrate, and phosphoric acid were applied in solution in amounts equivalent to 100 pounds per acre of potassium, nitrogen, and phosphorus, respectively. Seven treatments, N, P, K, NP, NK, KP, and NPK, and no treatment were assigned at random to eight plots in each of eight blocks in one long nursery bed. The entire lay-out was then quartered and peat applied at the rate of about 0.2 pound per square foot to two of the quarters, randomly selected. Each of the four quarters was then halved and 2 gallons of a turbid solution of calcium hydroxide applied to each of four of the eight halves, randomly selected.

Counts were made in July, and counts and height measurements were made in September. Both sets of data are presented in table 1. No significant effect of peat on height growth or density of seedlings appeared in this experiment, hence averages according to application of peat are not included in table 1.

TABLE 1.—*Average density in July and September 1937, of pitch pine seedlings in Chillicothe nursery plots, seeded in the spring of 1937*

Fertilizer [1]	Average seedling density per square foot					Average seedling height [3] in September 1937
	July 1937			September 1937		
	Limed [2] plots	Unlimed plots	All plots	Limed [2] plots	Unlimed plots	
	Number	*Number*	*Number*	*Number*	*Number*	*Inches*
Nitrogen, phosphorus, and potassium	12.6	10.0	11.3	.8.4	8.7	1.9
Nitrogen and phosphorus	13.5	9.2	11.3	7.8	8.1	1.9
Nitrogen and potassium	27.0	8.3	17.6	18.4	6.6	1.3
Potassium and phosphorus	28.1	31.2	29.6	28.8	32.8	1.9
Nitrogen	20.1	11.8	16.0	11.1	8.8	1.3
Phosphorus	26.3	25.4	25.8	22.2	23.4	1.7
Potassium	19.7	30.0	24.8	27.4	29.4	1.3
None	21.1	24.8	23.0	19.7	23.5	1.3
Minimum mean difference significant at—						
5-percent level		8.8		12.4	7.05	.27
1-percent level		11.9		16.8	9.57	.37

[1] Applied at rate of 100 pounds of each element per acre.
[2] Turbid solution of calcium hydroxide applied at rate of 2 gallons to 12 square feet.
[3] Height measurements were made in thirtieths of a foot, converted to inches, and rounded off.

The outstanding observation in this experiment is the effect of nitrogen. For nitrogen and every fertilizer treatment including nitrogen, seedling density of unlimed plots averaged less than for no treatment. Neither potassium nor phosphorus alone influenced density on unlimed plots significantly as measured in July, but association of increased density with potassium was indicated by the September counts on both limed and unlimed plots. Phosphorus and potassium together caused the highest density recorded in the experiment. Where nitrogen was added to phosphorus or to the potassium-phosphorus combination, density was reduced. Peat did not appreciably increase density, neither did it reduce it. The same is true of lime alone, but the interaction of lime and some fertilizer treatments did influence density. Lime reduced the injurious effect of nitrogen. Immediately after addition of lime to soil treated with ammonium nitrate the odor of ammonia was noticed, indicating liberation of some ammonia and consequent reduction of total quantity of nitrogen.

When seedlings were measured in September, it was not observed that nitrogenous fertilizers in conjunction with soil amendments had had any injurious effects on height growth.

The nitrogen-phosphorus combination gave superior height, although it gave the lowest fall density on limed plots (table 1). Potassium alone did not increase height significantly, but the combination of potassium and phosphorus and that of nitrogen, potassium, and phosphorus did. Apparently the increase in height growth was associated with phosphorus. This soil had an abundance of red oxide of iron, but either rigid fixation of phosphorus by iron did not occur or the soil was sufficiently alkaline to render any iron phosphate so formed relatively soluble and thus available to pine seedlings.

VALLONIA NURSERY

At the Vallonia nursery an experimental seeding of shortleaf pine (*Pinus echinata*) was made in the fall of 1937 to test response to (1) some of the minor elements, (2) potassium and phosphorus at normal levels of application, (3) an excessive quantity of an organic nitrogenous fertilizer, (4) three levels of phosphorus, (5) phosphorus at three levels of soil reaction, and (6) peat incorporated in the surface soil. The lay-out consisted of 2 blocks of 39 plots each. One block received peat at the rate of about 0.2 pound per square foot (4 tons per acre), the other none. Each of these large blocks was divided into 3 smaller blocks of 13 plots each, to which the following treatments were assigned at random: Copper sulfate, manganese sulfate, magensium sulfate, zinc sulfate, and boric acid each at the rate of 60 pounds of the element per acre; potassium sulfate at the rate of 120 pounds of potassium per acre; soybean meal at the rate of 400 pounds of nitrogen per acre; treble superphosphate at the rate of 100 pounds of phosphorus per acre for each of three levels of reaction (pH 4, 6, and 8); treble superphosphate at the rate of 40 and 500 pounds of phosphorus per acre in soil at pH 6; and no treatment.

Introduction of a more efficient sprinkling system at the Vallonia nursery in 1937 made unnecessary any further treatment to prevent concentration of lime salts in the surface soil.

The seedlings were counted in May of 1938 and were counted and measured for height early in September of that year; the results are presented in table 2. In the spring of 1939 they were lifted, washed, measured, and weighed. The results are presented in table 3.

TABLE 2.—*Average measurements in May 1938 and in the fall of 1938, of short-leaf pine seedlings in Vallonia nursery plots, seeded in the fall of 1937*

Fertilizer and rate of application per acre	Soil reaction	Average seedling density per square foot in May 1938 on plots having—		Average seedling measurements in fall of 1938 on plots having—			
				Peat		No peat	
		Peat	No peat	Density per square foot	Height	Density per square foot	Height
	pH	*Number*	*Number*	*Number*	*Inches*	*Number*	*Inches*
1. Copper sulfate, to give 60 pounds Cu	6	71	35	10.8	4.8	4.2	3.3
2. Manganese sulfate, to give 60 pounds Mn	6	73	34	12.9	5.5	4.8	2.8
3. Magnesium sulfate, to give 60 pounds Mg	6	69	35	8.8	4.8	6.1	3.0
4. Zinc sulfate, to give 60 pounds Zn	6	41	29	12.8	5.8	3.4	2.7
5. Potassium sulfate, to give 120 pounds K	6	43	40	11.3	6.5	6.4	3.2
6. Boric acid, to give 60 pounds B	6	44	52	9.4	4.8	6.7	3.7
7. Soybean meal, to give 400 pounds N	6	7	4	1.7	4.9	1.0	.8
8. Treble superphosphate, to give 100 100 pounds P	4	50	25	9.1	7.7	4.5	7.4
9. Treble superphosphate, to give 100 pounds P	8	37	16	5.7	5.2	2.1	3.4
10. Treble superphosphate, to give 40 pounds P	6	49	34	7.2	6.7	4.5	5.8
11. Treble superphosphate, to give 100 pounds P	6	62	26	13.5	7.4	4.4	7.2
12. Treble superphosphate, to give 500 pounds P	6	64	32	13.8	7.9	4.1	9.4
13. None	6	83	28	10.3	4.9	2.3	2.4
Minimum mean difference significant at—							
5-percent level		44	27	5.0	1.62	3.8	1.46
1-percent level		61	37	8.5	2.19	5.3	2.06

TABLE 3.—*Average measurements in the spring of 1938, of shortleaf pine seedlings in Vallonia nursery plots, seeded in the fall of 1937*

Fertilizer and rate of application per acre	Soil reaction	Average seedling measurements in spring of 1939 on plots having—											
		Peat						No peat					
		Length		Green weight		Dry weight		Length		Green weight		Dry weight	
		Root	Top	Root	Top	Root	Top	Root	Top	Root	Top	Root	Top
	pH	*In.*	*In.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*In.*	*In.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1. Copper sulfate, to give 60 pounds Cu	6	13.7	8.6	6.0	10.5	2.11	3.07	10.4	5.5	3.3	5.6	0.97	1.38
2. Manganese sulfate, to give 60 pounds Mn	6	12.9	8.2	5.5	9.8	1.74	2.59	8.9	3.8	1.6	2.4	.56	.66
3. Magnesium sulfate, to give 60 pounds Mg	6	12.9	8.3	5.5	10.3	1.72	2.64	12.0	6.8	6.2	11.6	1.85	2.83
4. Zinc sulfate, to give 60 pounds Zn	6	12.4	7.2	5.6	9.1	1.77	2.33	11.8	7.5	7.0	11.8	1.82	2.94
5. Potassium sulfate, to give 120 pounds K	6	12.1	6.4	3.7	6.1	1.23	1.56	11.1	5.9	4.7	9.1	1.44	2.26
6. Boric acid, to give 60 pounds B	6	10.1	6.5	2.8	4.9	.96	1.40	10.1	5.4	2.7	4.8	.89	1.34
7. Soybean meal, to give 400 pounds N	6	12.1	6.9	3.6	5.6	1.08	1.58	12.4	7.1	7.1	9.3	2.08	3.04
8. Treble superphosphate, to give 100 pounds P	4	13.0	7.3	4.7	8.3	1.48	2.18	14.2	9.0	14.9	32.3	4.83	8.72
9. Treble superphosphate, to give 100 pounds P	8	14.0	7.1	6.7	10.5	2.15	2.79	10.2	4.9	2.6	4.5	.80	1.04
10. Treble superphosphate, to give 40 pounds P	6	12.8	6.2	5.0	7.1	1.59	1.94	9.5	5.1	4.1	7.5	1.35	1.94
11. Treble superphosphate, to give 100 pounds P	6	11.8	7.5	3.7	7.4	1.24	2.08	11.0	5.9	3.8	6.9	1.16	1.64
12. Treble superphosphate, to give 500 pounds P	6	12.4	7.0	4.9	8.0	1.51	2.01	11.5	6.1	6.1	11.3	1.85	2.80
13. None	6	13.1	7.7	5.1	8.9	1.72	2.56	11.2	6.9	3.0	5.1	.91	1.46
Minimum mean difference significant at—													
5-percent level		2.30	2.56	3.62	6.72	1.19	1.88	3.78	3.62	8.20	17.6	2.58	4.77
1-percent level		3.14	3.52	4.92	9.20	1.63	2.56	5.16	4.92	11.20	24.1	3.53	6.52

Analysis showed no covariance between height and density. In other words, in this experiment the seedling density was not great enough to affect height growth. .

Application of peat resulted in greater density. In September 1938 the height of the seedlings grown in peat-treated soils was greater to a highly significant degree than that of the seedlings grown in soils without peat. When the seedlings were lifted and measured in the spring of 1939, those grown on the peat-treated plots showed a highly significant superiority both in root and top length.

No evidence was found that the mineral salts had caused damage. Perhaps this is due to the fact that they were applied in the fall and were subjected to leaching all winter. It seems likely, however, that the soil absorbed and held most of the soluble cations—although in this light-textured soil the mineral cations probably were diffusely distributed. Injury was less than that on heavier soils reported by some investigators. So far as height growth is concerned, copper, manganese, magnesium, and boron were all ineffective. Density, however, was found in September 1938 to be significantly greater on the plots treated with magnesium and boron than on the untreated plots.

One of the most interesting observations was the effect of soybean meal on density and height. Although application of ammonium nitrate in previous experiments at Chillicothe had resulted in high mortality of seedlings, it had been expected that application of this organic nitrogenous material would not result in injury. However, nitrates rapidly liberated from the soybean meal caused excessive loss of seedlings. The soil surface became grayish green in color from algal growth, and loss from damping-off was very severe.

The effects of the 100-pound application of phosphorus on seedling height and density in September 1938 varied somewhat. On the peat-treated plots phosphorus applied to soil at pH 8 gave poor results both as to density and height. On the no-peat plots, height growth where phosphate was applied at pH 8 was greatly inferior to that where phosphate was applied at pH 4 or 6. Trees on soil at pH 6 not treated for soil reaction responded to phosphorus almost as well as did those on soil at pH 4. This indicates that there was effective phosphorus fixation at pH 8 and none at either pH 4 or pH 6.

In the test of superphosphate at 40, 100, and 500 pounds phosphorus per acre, the 100-pound application did not result very differently from the 40, except in regard to seedling density in September. The 500-pound application resulted in greater seedling height than the 100-pound application on plots not treated with peat, but did not give any superior results elsewhere.

The variations with fertilizer treatment shown by heights recorded early in September of the second year had largely disappeared by the following March, except for plots treated with treble superphosphate at pH 4. Both root and top weights (green and dry) of seedlings on plots phosphate-treated at pH 4 were superior to all others by highly significant differences. The superiority of seedling height on peat-treated soil recorded in September 1938 had not become obscured by differential growth in March 1939. The grand averages for length of tops in September and March, respectively, were 5.9 and 7.8 inches on plots with peat and 4.3 and 6.6 inches on plots without peat.

In the Vallonia as in the Chillicothe nursery, results of the experiment set up in 1937 indicated clearly that acid phosphates stimulated height growth of pines and nitrogen applied at seeding time reduced seedling density. Potassium was probably ineffective.

Root-top ratios for length and for green and dry weights at the time the seedlings were lifted in March of 1939 are presented in table 4. No significant differences according to fertilizer treatment or soil amendment appeared in these ratios. Peat increased height growth significantly root length proportionately.

TABLE 4.—*Average root-top ratios of shortleaf pure seedlings in the spring of 1939 in Vallonia plots, seeded in the fall of 1937*

Fertilizer and rate of application per acre	Soil reaction	Average root-top ratios in spring of 1939 for plots having—					
		Peat			No peat		
		Length	Green weight	Dry weight	Length	Green weight	Dry weight
	pH	*Inches*	*Grams*	*Grams*	*Inches*	*Groms*	*Grams*
1. Copper sulfate, to give 60 pounds Cu	6	1.6	0.57	0.68	1.9	0.60	0.73
2. Manganese sulfate, to give 60 pounds Mn	6	1.6	.56	.67	2.4	.58	.78
3. Magnesium sulfate, to give 60 pounds Mg	6	1.6	.54	.66	1.8	.56	.68
4. Zinc sulfate, to give 60 pounds Zn	6	1.8	.61	.75	1.6	.59	.66
5. Potassium sulfate, to give 120 pounds K	6	2.0	.63	.80	2.0	.59	.75
6. Boric acid, to give 60 pounds B	6	1.8	.60	.73	2.0	.58	.69
7. Soybean meal, to give 400 pounds N	6	1.8	.65	.68	1.8	1.11	.72
8. Treble superphosphate, to give 100 pounds P	4	1.8	.59	.71	1.6	.51	.61
9. Treble superphosphate, to give 100 pounds P	8	2.0	.65	.81	2.1	.58	.77
10. Treble superphosphate, to give 40 pounds P	6	2.1	.69	.81	2.0	.52	.69
11. Treble superphosphate, to give 100 pounds P	6	1.7	.58	.68	1.9	.55	.72
12. Treble superphosphate, to give 500 pounds P	6	1.8	.61	.76	2.0	.55	.69
13. None	6	1.7	.60	.72	1.8	.60	.65
Average		1.8	.61	.73	1.9	.61	.70
Minimum mean difference (for values for single treatments) significant [1] at—							
5-percent level		1.8	.34	.20	1.8	.34	.20
1-percent level		2.4	.46	.26	2.4	.46	.26

[1] Values were calculated on the basis of complete data, not of averages.

1938 EXPERIMENTS

Since in 1937 only one form of fertilizer was used for each nutrient element, a question arose as to the probable effectiveness of other carriers. Further tests of peat were needed because response to peat had not been the same on the Vallonia as on the Chillicothe nursery soil. Further study of the influence of soil reaction, also, was needed.

In the spring of 1938 a fertilizer experiment was started at each of the three nurseries to test the effectiveness of five kinds of phosphatic material—treble superphosphate, rock phosphate, iron phosphate, aluminum phosphate, and liquid phosphoric acid. Aluminum phosphate was applied at Chillicothe only and iron phosphate at Vallonia only. Tests were made also of four kinds of nitrogenous

material—ammonium sulphate, sodium nitrate, dried blood, and soybean meal—and of three kinds of potassic material—potassium sulphate, tobacco stems, and green sand marl. Each of the three series of materials containing phosphorus, nitrogen, and potassium, respectively, was randomized completely in the nursery beds. In addition, peat was applied on randomly selected phosphate and nitrogen plots at Chillicothe and Vallonia. Soil reaction was varied by applying finely ground limestone or sulfuric acid. All tests were replicated. The experimental design provided an opportunity for evaluating each fertilizing material individually, without any attempt to study nutrient combinations.

TABLE 5.—*Average density and height in September 1938, of pitch pine seedlings treated with different fertilizer materials and soil amendments, in plots at Chillicothe, seeded in the spring of 1938*

Fertilizer and rate of application per acre	Average seedling measurements in September 1938.	
	Density per square foot	Height
Phosphorus:	*Number*	*Inches*
Aluminum phosphate, to give 400 pounds P	9	2.90
Rock phosphate, to give 400 pounds P	11	1.57
Treble superphosphate, to give 100 pounds P	10	3.22
Liquid phosphoric acid, to give 100 pounds P	9	2.79
None	11	1.72
Minimum mean difference significant at—		
5-percent level	1.9	.37
1-percent level	2.54	.50
Nitrogen:		
Soybean meal, to give 100 pounds N	7	1.50
Dried blood, to give 100 pounds N	4	1.40
Ammonium sulfate, to give 100 pounds N	2	1.36
Sodium nitrate, to give 100 pounds N	8	1.60
None	9	1.58
Minimum mean difference significant at—		
5-percent level	3.6	.89
1-percent level	5.2	.57
Potassium:		
Potassium sulfate, to give 100 pounds K	12	1.42
Tobacco stems, to give 100 pounds K	7	1.20
Greensand marl, to give 500 pounds K	9	1.40
None	5	1.08
Minimum mean difference significant at—		
5-percent level	4.1	.45
1-percent level	6.0	.66
Lime on phosphate plots (ground limestone):		
2 tons	8	2.26
None	12	2.62
Minimum mean difference significant at—		
5-percent level	1.2	.20
1-percent level	1.6	.32
Peat on phosphate plots:		
4 tons	10	2.64
None	10	2.24
Minimum mean difference significant at—		
5-percent level	1.2	.20
1-percent level	1.6	.32

The materials were applied at the following rates per acre: Rock phosphate, iron phosphate, and aluminum phosphate, 400 pounds phosphorus; nitrogenous substances, 100 pounds nitrogen; potassium

sulfate and tobacco stems, 100 pounds potassium; greensand marl, approximately 500 pounds potassium; peat, 4 tons; and calcium carbonate, 2 tons. At Chillicothe and at Vallonia, soil reaction was adjusted to the two pH levels 4 and 7 (or slightly higher) by use of sulfuric acid and limewater, respectively. All soil amendments and fertilizer applications were made at seeding time. Pitch pine seed was used at Chillicothe and Vallonia, shortleaf pine seed at Licking.

CHILLICOTHE NURSERY

Seedling counts and height measurements were taken in the Chillicothe nursery early in September of 1938, with the results presented in table 5. Height growth on the plots treated with rock phosphate did not differ significantly from that on the check plots, but liquid phosphoric acid, aluminum phosphate, and treble superphosphate gave significantly greater seedling heights. Aluminum phosphate is ordinarily considered unavailable, but here, probably because of the relatively high reaction of the soil, it was as effective as liquid phosphoric acid. Liquid phosphoric acid and aluminum phosphate caused lower seedling density; this is attributed to natural thinning induced by increased growth. Density was drastically reduced by ammonium sulfate and dried blood. Dried blood is not immediately available, but nitrifies rapidly in warm moist soil. Injury from dried blood was not considered "burning," but was thought to have resulted from attacks of damping-off fungi that had been stimulated by the rapid evolution of nitrates. Peat increased seedling height where used with phosphorus, but did not do so where used with nitrogen or potassium. Lime caused lower density on phosphate plots.

Seedling density on the plots treated with potassium sulfate was greater than that on the check plots.

VALLONIA NURSERY

At the Vallonia nursery, seedling counts were taken in June 1938 and both density and height were measured in September of that year, with the results presented in table 6. In the spring of 1939 the seedlings on the sample strips were lifted and washed, measured, and weighed.

In the June counts no significant effects on seedling density were noted on the phosphate plots. A highly significant difference in density appeared between the nitrogen plots and the check plots, and between the limed and the unlimed plots. Potassium fertilizers had little or no effect on density. When counts and height measurements were made in September, no significant differences in either density or height were found that could be attributed to application of phosphorous, nitrogen, or potassium. When the seedlings were lifted in the spring of 1939, no significant differences in root length, top length, or root or top green or dry weight resulting from nitrogen or potassium additions were noted; but highly significant differences from check-plot values in length of both roots and tops and in green and dry weights were recorded for all plots treated with prosphatic materials other than rock phosphate (table 7). On the nitrogen-treated plots lime decreased top length, but did not affect root length or green or dry weight of roots or tops. Root-top length ratios for seedlings grown on nitrogen plots are given in table 8. On the phosphorus-

treated plots lime did not affect root or top length; it did reduce the green and dry weights of both roots and tops. Peat had no significant effects.

TABLE 6.—*Average density in June and September 1938 and average height in September 1938 of pitch pine seedlings treated with different fertilizer materials and soil amendments, in plots at Vallonia, seeded in the spring of 1938*

Fertilizer and rate of application per acre	Average seedling density per square foot		Average seedling height in September 1938
	June 1938	September 1938	
Phosphorus:	*Number*	*Number*	*Inches*
Iron phosphate, to give 400 pounds P	65	11	4.1
Rock phosphate, to give 400 pounds P	74	16	3.9
Treble superphosphate, to give 100 pounds P	70	16	3.8
Liquid phosphoric acid, to give 100 pounds P	65	12	4.0
None	79	12	4.0
Minimum mean difference significant at—			
5-percent level	19	6	.7
1-percent level	25	9	.9
Nitrogen:			
Soybean meal, to give 100 pounds N	8	4.0	2.6
Dried blood, to give 100 pounds N	12	2.6	2.4
Ammonium sulfate, to give 100 pounds N	32	3.6	2.1
Sodium nitrate, to give 100 pounds N	20	4.6	1.8
None	64	2.9	2.0
Minimum mean difference significant at—			
5-percent level	13	8	3.4
1-percent level	18	11	4.6
Potassium:			
Potassium sulfate, to give 100 pounds K	50	16	1.8
Tobacco stems, to give 100 pounds K	61	8	1.8
Greensand marl, to give 500 pounds K	57	13	2.0
None	65	5	1.6
Minimum mean difference significant at—			
5-percent level	52	19	.6
1-percent level	75	27	.8
Lime [1] (ground limestone):			
2 tons	19	2.8	3.6
None	39	4.3	4.0
Minimum mean difference significant at—			
5-percent level	12	4.0	.5
1-percent level	16	5.0	.6

[1] Values presented are for plots on which phosphorus was applied.

TABLE 7.—*Average measurements in the spring of 1939, of pitch pine seedlings treated with different phosphatic materials, in plots at Vallonia, seeded in the spring of 1938*

Fertilizer and rate of application per acre	Average seedling measurements in spring of 1939					
	Length		Green weight		Dry weight	
	Root	Top	Root	Top	Root	Top
	Inches	*Inches*	*Grams*	*Grams*	*Grams*	*Grams*
Iron phosphate, to give 400 pounds P	11.4	6.0	2.72	4.74	0.80	1.40
Rock phosphate, to give 400 pounds P	10.2	5.0	1.70	2.96	.46	.82
Treble superphosphate, to give 100 pounds P	11.1	6.1	2.61	4.78	.73	1.35
Liquid phosphoric acid, to give 100 pounds P	11.6	6.1	2.61	5.08	.86	1.46
None	10.4	5.2	1.80	3.06	.46	.86
Minimum mean difference significant at—						
5-percent level	.6	.6	.49	.98	.14	.26
1-percent level	.7	.8	.65	1.31	.19	.35

TABLE 8.—*Average root-top length ratios in the spring of 1939 of pitch pine seedlings treated with nitrogen, in plots at Vallonia, seeded in the spring of 1938*

Fertilizer and rate of application per acre	Average root-top length ratios in spring of 1939 for plots having—	
	Lime	No lime
Soybean meal, to give 100 pounds N	2.59	1.91
Dried blood, to give 100 pounds N	2.47	2.25
Ammonium sulfate, to give 100 pounds N	2.26	2.31
Sodium nitrate, to give 100 pounds N	2.65	1.88
None	2.14	2.13
Minimum mean difference significant at—		
5-percent level	0.58	
1-percent level	.79	
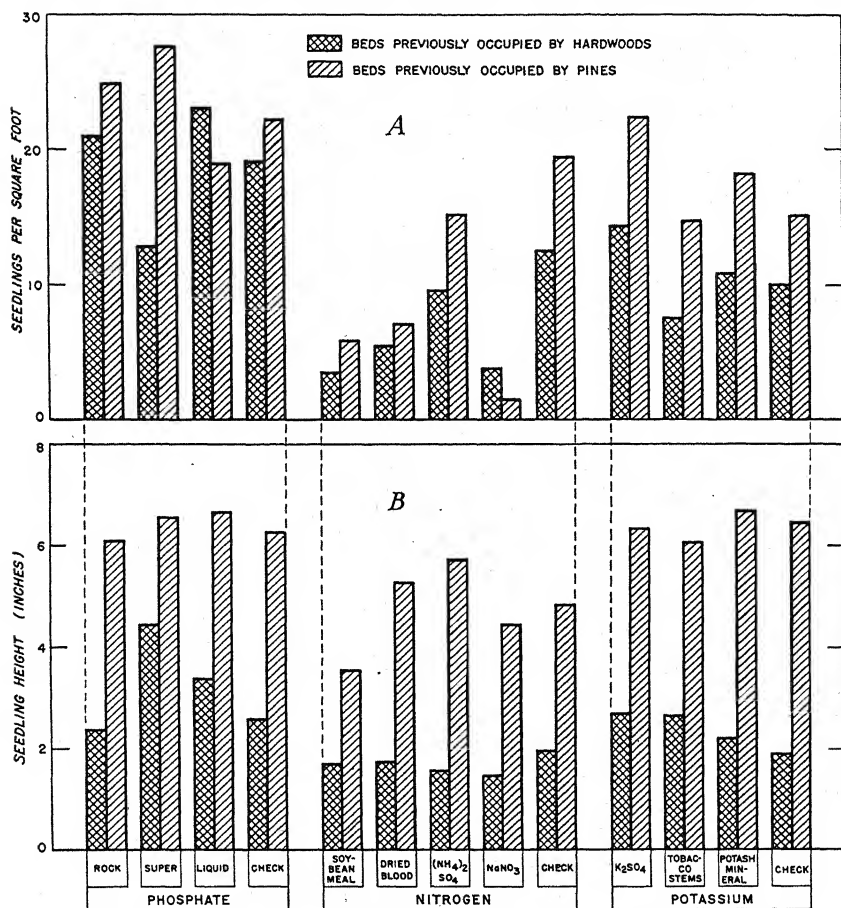
FIGURE 3.—Density (*A*) and height (*B*) in October 1938 of shortleaf pine seedlings on uninoculated and inoculated Licking plots, seeded in the spring of that year, treated with three kinds of phosphatic, four kinds of nitrogenous, and three kinds of potassic fertilizers. The fertilizers used, and rates of application per acre, were as follows: Rock phosphate, to give 400 pounds P; treble superphosphate, to give 100 pounds P; liquid phosphoric acid, to give 100 pounds P; soybean meal, to give 600 pounds N; dried blood, to give 100 pounds N; ammonium sulfate, to give 100 pounds N; sodium nitrate, to give 100 pounds N; potassium sulfate, to give 100 pounds K; tobacco stems, to give 100 pounds K; and potash mineral, to give 400 pounds K.
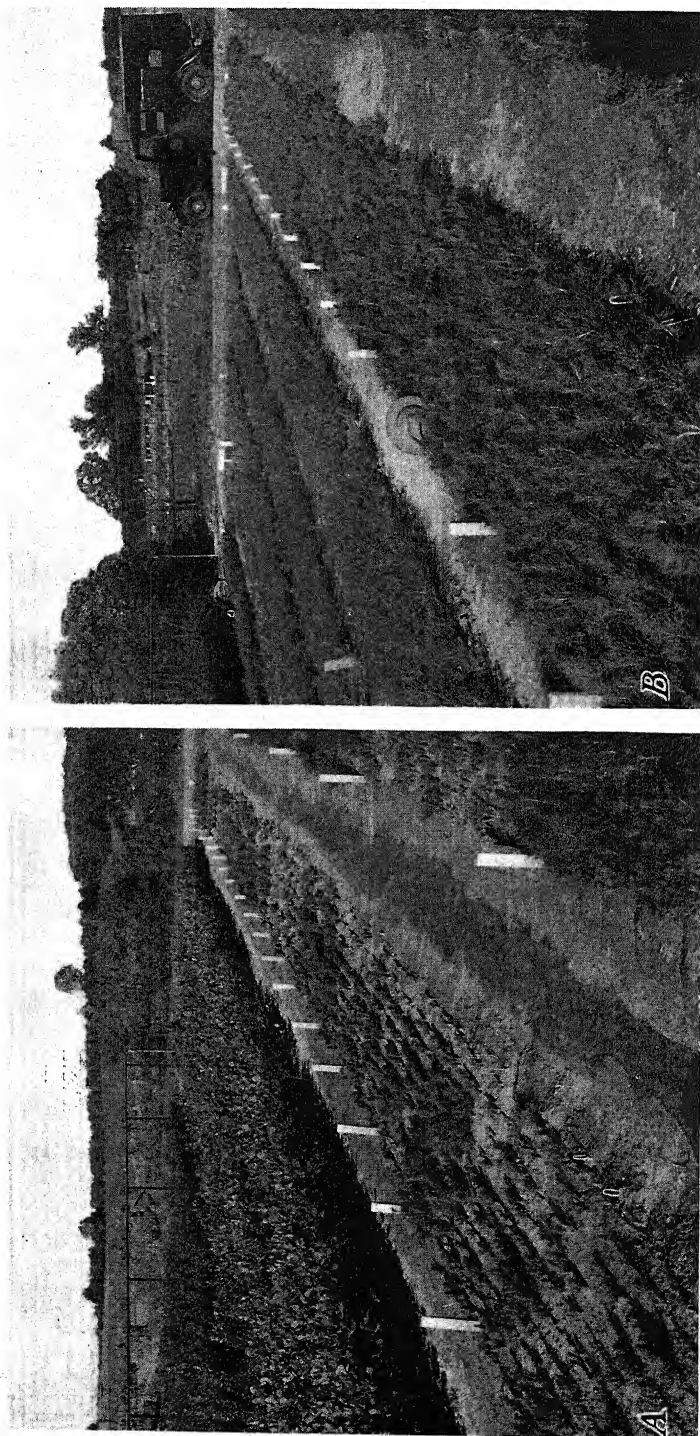
FIGURE 4.—Shortleaf pine seedbeds in Licking nursery of which one (A) had previously been in hardwoods and the other (B) had previously been in pines. These beds were seeded the same day and received identical treatment. The taller of the seedlings shown in A are on phosphate-treated plots.

LICKING NURSERY

In the Licking nursery the 1938 experiment differed from those of the same year at Chillicothe and Vallonia in that soil reaction was not altered, shortleaf pine was used instead of pitch pine, and plots were established by identical methods on soil previously in hardwoods and on soil previously in pines. The plot series on hardwood and pine soil will be referred to here as the hardwood-soil series and the pine-soil series. In October 1938 the seedlings on the sample strips were counted and measured for height. In the spring of 1939 they were lifted and washed, measured, and weighed. The results of the fall and the spring measurements are given in tables 9 and 10, and those of the fall measurements are presented in figure 3.

TABLE 9.—*Average measurements in the fall of 1938 of shortleaf pine seedlings treated with fertilizer materials and soil amendments, in plots at Licking, seeded in the spring of 1938*

|	Average seedling measurements in October 1938			
Fertilizer and rate of application per acre	Hardwood-soil series		Pine-soil series	
	Density per square foot	Height	Density per square foot	Height
Phosphorus:	*Number*	*Inches*	*Number*	*Inches*
Rock phosphate, to give 400 pounds P	20.8	2.4	24.8	6.1
Treble superphosphate, to give 100 pounds P	12.9	4.5	27.6	6.6
Liquid phosphoric acid, to give 100 pounds P	23.0	3.4	19.1	6.7
None	19.2	2.6	22.1	6.3
Minimum mean difference significant at—				
5-percent level	5.1	.8	6.8	.5
1-percent level	7.1	1.2	9.4	.8
Nitrogen:				
Soybean meal, to give 600 pounds N	3.6	1.7	5.8	4.9
Dried blood, to give 100 pounds N	5.3	1.8	7.2	4.5
Ammonium sulfate, to give 100 pounds N	9.6	1.6	15.2	5.8
Sodium nitrate, to give 100 pounds N	3.9	1.5	1.6	4.5
None	12.6	2.0	19.6	4.9
Minimum mean difference significant at—				
5-percent level	6.5	.25	2.8	1.1
1-percent level	8.9	.34	3.9	1.5
Potassium:				
Potassium sulfate, to give 100 pounds K	14.5	2.7	22.4	6.4
Tobacco stems, to give 100 pounds K	7.6	2.7	14.8	6.1
Potash mineral, to give 400 pounds K	10.8	2.2	18.2	6.7
None	10.0	1.9	15.2	6.5
Minimum mean difference significant at—				
5-percent level	6.6	.7	4.7	1.4
1-percent level	10.1	1.1	7.1	2.1
Peat on phosphorus plots:				
4 tons	20.0	3.5	26.0	6.6
None	19.0	2.9	21.0	6.2
Minimum mean difference significant at—				
5-percent level	4.0	.6	4.8	.39
1-percent level	5.0	.8	6.7	.54
Peat on nitrogen plots:				
4 tons	7.0	1.8	13.0	5.0
None	7.0	1.7	7.0	4.8
Minimum mean difference significant at—				
5-percent level	.41	.16	1.8	.7
1-percent level	.57	.22	2.4	.9

The outstanding observation in this 1938 Licking experiment was the superiority of seedlings grown on soil previously in pines as compared with those grown on other soil (fig. 4). When the seedlings

on the sample strips were lifted it was found that the roots of those grown after pines had many short, fleshy gray rootlets, but that the roots of those grown in soil not previously occupied by pines were brown and wiry, without short fleshy rootlets.

TABLE 10.—*Average measurements in the spring of 1939, of shortleaf pine seedlings treated with fertilizer materials and soil amendments, in plots at Licking, seeded in the spring of 1938*

Fertilizer and rate of application per acre	Average seedling measurements in spring of 1939									
	Hardwood-soil series				Pine-soil series					
	Length		Green weight		Length		Green weight		Air-dry weight	
	Root	Top	Root	Top	Root	Top	Root	Top	Root	Top
Phosphorus:	*Inches*	*Inches*	*Grams*	*Grams*	*Inches*	*Inches*	*Grams*	*Grams*	*Grams*	*Grams*
Rock phosphate, to give 400 pounds P	8.60	2.88	0.75	1.25	10.41	6.35	3.70	5.70	1.38	1.94
Treble superphosphate, to give 100 pounds P	10.85	5.50	2.75	4.48	10.07	6.90	3.66	5.88	1.40	2.06
Liquid phosphoric acid, to give 100 pounds P	9.71	4.02	1.42	2.16	10.58	7.00	4.40	7.60	1.55	2.56
None	8.55	2.94	1.00	1.60	10.80	7.10	4.32	6.87	1.63	2.36
Minimum mean difference significant at—										
5-percent level	.85	.94	.55	1.26	1.03	.99	.73	1.37	.36	.43
1-percent level	1.18	1.30	.76	1.74	1.43	1.38	1.02	1.90	.49	.60
Nitrogen:										
Soybean meal, to give 100 pounds N					9.93	5.60	4.35	5.85	1.50	1.98
Dried blood, to give 100 pounds N					10.20	5.58	4.52	6.20	1.70	2.21
Ammonium sulfate, to give 100 pounds N					10.47	5.67	4.20	5.98	1.55	2.08
Sodium nitrate, to give 100 pounds N					10.90	6.04	4.88	6.68	1.80	2.27
None					10.20	5.50	3.83	5.42	1.48	1.94
Minimum mean difference significant at—										
5-percent level					1.13	.71	1.09	1.50	.37	.51
1-percent level					1.55	.97	1.50	2.06	.51	.70
Potassium:										
Potassium sulfate, to give 100 pounds K	7.16	2.46	1.08	1.42	10.30	6.23	3.50	5.30	1.33	1.82
Tobacco stems, to give 100 pounds K	8.00	3.23	1.51	2.04	11.10	6.23	3.96	5.80	1.50	1.97
Greensand marl, to give 100 pounds K	6.07	2.73	.97	1.42	10.23	6.47	3.57	6.00	1.52	2.04
None	7.03	2.80	1.22	1.66	10.60	6.17	3.93	6.33	1.48	2.15
Minimum mean difference significant at—										
5-percent level	2.04	.58	.93	.95	.75	.72	.83	1.82	.51	.82
1-percent level	2.91	.82	1.41	1.42	1.13	1.09	1.14	2.51	.78	1.25
Peat:[1]										
4 tons			1.61	2.50						
None			1.34	2.25						
Minimum mean difference significant at—										
5-percent level			.39	.89						
1-percent level			.54	1.23						

[1] Values presented are for plots on which phosphorus was applied.

Measurements in the fall of the first year showed that superphosphate and liquid phosphoric acid had increased height growth in seedlings on hardwood soil. Lower seedling density was associated

with superphosphate treatment. Phosphate did not influence seedling height on pine soil. Retarded height growth and lower seedling density were associated with the soybean meal, ammonium sulfate, and sodium nitrate treatments on hardwood soil. On pine soil none of the nitrogenous fertilizer treatments affected seedling height, but all of them reduced density. Only in one instance did potassium affect seedlings materially in this experiment; higher density was associated with potassium sulfate on pine soil.

Where phosphorus was applied in 1938, peat had little or no effect on either density or height on hardwood soil, but increased density and height on pine soil. In the nitrogen tests on hardwood soil peat did not increase either density or height, but in those on pine soil it increased density.

When lifted in the spring of 1939, the seedlings grown on some hardwood-soil plots showed beneficial effects from the application of phosphates. Those from plots treated with liquid phosphoric acid were superior in top and root length and top weight, and those from plots treated with treble superphosphate were superior in both length and weight of tops and roots. The seedlings on hardwood-soil plots treated with nitrogenous fertilizers failed completely in 1939; not enough remained for an adequate sample. Similar mortality did not occur on pine-soil plots. There was no evidence that the seedlings on pine soil had been affected in any way by any of the fertilizers applied.

1939 EXPERIMENTS

When on completion of the 1938 experiments it became evident that phosphorus was the most effective fertilizing element, and that nitrogen in any form applied at seeding time greatly lowered pine seedling density, it was decided to test application of nitrogenous fertilizers after seeding. A combination experiment was designed for three levels each of phosphorus, nitrogen, and potassium, applied as treble superphosphate, ammonium sulfate, and potassium sulfate. These levels, with the times of application, were: Phosphorus—none, 40 pounds at seeding time, and 100 pounds at seeding time; nitrogen—none, 25 pounds 2 weeks after seedling emergence, and 25 pounds 4 weeks after emergence; potassium—none, 25 pounds 2 weeks after emergence, and 25 pounds 4 weeks after emergence. Three experimental blocks were established, each containing twenty-seven 3- by 4-foot plots representing the 27 combinations of the 3 mineral nutrients at 3 levels each, completely randomized.

In addition to this test of nitrogen and potassium, a further test was made of rock phosphate and greensand marl on soils differing in reaction. It was thought possible that failure to get results with these materials previously had been caused by unfavorable soil reaction. On one-third of the plots receiving rock phosphate or greensand marl, calcium carbonate was added in excess in order to raise the reaction to the neutral point. On another third, sufficient dilute sulphuric acid was sprinkled to reduce the soil reaction to about pH 4. On the remainder the soil reaction, approximately pH 6, was not altered.

This experimental design was used at each of the three nurseries, with shortleaf pine. At Chillicothe more than half the seedlings

died en bloc from causes independent of treatment; therefore this part of the experiment was abandoned.

In the spring of 1940, at Vallonia and Licking, the seedlings on the sample strips were lifted, washed, measured, and weighed green. The average root and top lengths and weights for the phosphorus-nitrogen-potassium experiment are given in table 11 and those for the rock phosphate-greensand marl experiment in table 12.

Association of greater lengths of roots and tops with phosphate treatment was consistent throughout the 1939 experiments. It should be noted that 100 pounds of phosphorus per acre gave better results than 40 pounds. Nitrogen increased root and top length at Vallonia and reduced top length at Licking. It increased root and top weight at Vallonia but not at Licking. At Vallonia the seedlings on plots treated with nitrogenous salts 2 weeks after seedling emergence were superior in top length and in root and top weights to those on plots not so treated; and seedlings on plots similarly treated 4 weeks after emergence were superior in all measurements. At Licking, ammonium sulfate applied 2 weeks after seedling emergence had no effect on root or top length or weight and the same application 4 weeks after emergence was ineffective, except that it reduced top length. In no case did potassium influence growth.

Some beneficial results from the application of rock phosphate were indicated, but they did not rise to significant levels. No beneficial results could be found from the use of greensand marl on soil at pH 4, 6, or 7, in either nursery. A combination of rock phosphate and greensand marl gave a highly significant increase in top weight at Licking in soil at pH 6.

TABLE 11.—*Average measurements in the spring of 1940, of seedlings treated with phosphorus, nitrogen, and potassium, in plots at Vallonia and Licking, seeded in the spring of 1939*

Fertilizer treatment [1]	Vallonia					Licking				
	Average density per square foot	Average length		Average green weight		Average density per square foot	Average length		Average green weight	
		Root	Top	Root	Top		Root	Top	Root	Top
Phosphorus:	*Number*	*Inches*	*Inches*	*Grams*	*Grams*	*Number*	*Inches*	*Inches*	*Grams*	*Grams*
P₀	36	10.5	5.6	1.6	3.5	17	12.2	6.5	3.7	6.8
P₁	33	10.7	5.8	1.7	3.6	13	12.2	6.6	4.1	7.2
P₂	29	11.1	6.2	2.0	4.2	11	12.6	7.0	4.6	8.6
Nitrogen:										
N₀	34	10.5	5.4	1.6	3.2	13	12.6	6.9	4.2	7.8
N₁	32	10.8	6.2	1.9	4.2	13	12.4	6.8	4.3	7.7
N₂	33	10.9	6.0	1.9	4.0	13	12.1	6.4	4.0	7.1
Potassium:										
K₀	33	10.8	5.8	1.7	3.5	13	12.7	6.8	4.3	7.8
K₁	32	10.9	6.0	1.8	4.0	13	12.1	6.7	4.1	7.6
K₂	33	10.6	5.8	1.8	3.8	13	12.4	6.6	4.0	7.2
Minimum mean difference significant at—										
5-percent level	4.18	.4	.4	.2	.5	1.70	.6	.4	.4	.8
1-percent level	5.58	.5	.5	.3	.6	2.26	.8	.5	.6	1.1

[1] Key:
    Phosphorus—
        P₀=None.
        P₁=40 pounds at seeding time.
        P₂=100 pounds at seeding time.

Nitrogen and potassium—
    N₀ or K₀=None.
    N₁ or K₁=25 pounds per acre 2 weeks after seedling emergence.
    N₂ or K₂=25 pounds per acre 4 weeks after seedling emergence.

TABLE 12.—*Average measurements in the spring of 1940, of seedlings treated with rock phosphate, greensand marl, or a combination of the two, in plots at Vallonia and Licking, seeded in the spring of 1939*

Nursery and potassium treatment [1]	Length of seedlings grown in soil having a reaction of—						Green weight of seedlings grown in soil having a reaction of—					
	pH 4		pH 6		pH 7+		pH 4		pH 6		pH 7+	
	Root	Top	Root	Top	Root	Top	Root	Top	Root	Top	Root	Top
Vallonia:	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
RM	10.7	4.6	11.1	5.0	10.1	4.7	1.8	3.3	1.2	2.0	1.5	2.5
R	11.6	5.2	10.9	4.4	10.8	4.6	1.5	2.8	1.7	2.8	1.9	3.3
M	10.9	5.0	10.2	3.9	10.1	4.0	2.1	3.6	1.4	2.3	2.2	3.9
None	10.7	4.6	11.3	4.4	10.6	4.2	1.9	3.4	1.4	2.5	1.9	3.2
Minimum mean difference significant at—												
5-percent level	1.00	1.27	1.00	1.27	1.00	1.27	.76	1.70	.76	1.70	.76	1.70
1 percent level	1.36	1.73	1.36	1.73	1.36	1.73	1.04	2.31	1.04	2.31	1.04	2.31
Licking:												
RM	13.2	6.4	13.6	7.1	14.0	6.4	3.8	6.6	5.1	10.4	5.2	8.7
R	12.9	6.7	12.8	7.1	13.4	5.9	4.1	7.2	3.4	7.4	3.8	8.1
M	13.1	6.1	12.8	6.1	14.3	6.1	3.8	6.3	4.4	7.5	4.9	7.8
None	13.5	6.1	13.0	6.4	13.3	6.5	3.8	6.7	3.9	6.4	4.5	7.9
Minimum mean difference significant at—												
5-percent level	1.66	1.08	1.66	1.08	1.66	1.08	1.56	2.38	1.56	2.38	1.56	2.38
1-percent level	2.26	1.47	2.26	1.47	2.26	1.47	2.11	3.24	2.11	3.24	2.11	3.24

[1] Key: R = Rock phosphate, 4 tons per acre.
M = Greensand marl, 4 tons per acre.
RM = Combination of R and M.

Density was not affected by either nitrogen or potassium, but was reduced by phosphorus (table 11). At the Vallonia nursery, seedlings per square foot averaged 36, 33, and 29, respectively, for no phosphorus, 40 pounds phosphorus, and 100 pounds phosphorus per acre; and at the Licking nursery they averaged 17, 13, and 11, respectively, for the same applications. Although density varied inversely with quantity of phosphorus applied, height varied directly with it.

DISCUSSION

The requirement of light texture for easy workability places certain handicaps on the maintenance of nursery soils. Light-textured, well-drained soils have high oxidation rates, hence their organic-matter content usually is not only low at the time of nursery establishment but is likely to require the addition of large quantities of organic matter for its maintenance. It is doubtful whether the organic-matter content of very light soils can be permanently maintained by the use of cover crops, to say nothing of increasing it in this way.

Well disintegrated peat is probably the best means of adding organic matter to nursery soil. It resists oxidation and ameliorates soil structure. It also serves as a medium for holding acid, where acid must be applied to reduce the alkalinity of the soil. Peat helps to hold moisture in the upper soil, and insulates it against rapid evaporation. The beneficial effects of peat added to light nursery soils are indicated in the results of the experiments discussed here. Peat was particularly effective in the 1937 Vallonia tests, increasing both density and height of shortleaf pine seedlings. In the 1938 tests

it increased height of pitch pine on phosphate-treated plots at Chillicothe, increased density and height of shortleaf pine on phosphate-treated plots previously in pines at Licking, and increased density on nitrogen-treated plots previously in pines at Licking. Its effects were not entirely consistent, for in the 1938 tests it failed to cause any increase in seedling height or density at Vallonia, where it had been very effective in the 1937 tests. Perhaps this inconsistency was due to the more efficient sprinkling in 1938, which may have canceled any possible advantage from the water-holding capacity of the peat.

The light texture necessary for workability in a forest nursery soil increases the difficulty of maintaining an optimum nitrogen supply because rapid aeration causes rapid oxidation and evolution of nitrates from organic debris, and free drainage quickly removes soluble nitrates. Nitrification of large quantities of organic matter is injurious to pine seedlings during the germination and emergence period. All the forms of nitrogenous fertilizer used in these experiments reduced seedling density where applied at seeding time. Soluble nitrogenous fertilizers were most beneficial where applied about 2 weeks after seedling emergence. Soluble mineral nutrients of any kind are injurious unless applied in moderate quantities and quickly washed into the soil. Soybean meal in quantities smaller than those used in this study might have given beneficial results, but it seems probable that nitrates characteristically have an undesirable effect on pine seedlings in the early stages. Possibly soybean meal might be used to advantage as a surface dressing 2 weeks after seedling emergence. At that time it would be less likely to "burn" the seedlings or to increase damping-off through stimulation of soil fungi.

The fact that no form of potassic fertilizer increased pine seedling height on the soils studied does not mean that potassium is never beneficial to pine seedlings. The soils studied evidently were well supplied with potassium; this, however, is not true of some other soils in which pine seedlings are being or might be grown. Where potassium is needed it should be applied only in moderate quantities and after seedling emergence.

Pine seedlings have often been observed to fail even on soil containing ample mineral nutrients. The relation between mycorrhizae and seedling nutrition is as yet largely unexplained. Solubility of phosphates is known to vary with soil reaction. In acid soils well supplied with iron and aluminum phosphates, it is distinctly possible that fungi make these phosphates available by raising the soil reaction locally. On one soil not inoculated with mycorrhizal fungi, application of easily soluble phosphates increased thrift of pine seedlings. Application of such phosphates, however, did not raise this soil's production of pine seedlings to that of the same soil on an area where pines had been grown the previous year. A lag in development of some part of biotic balance has frequently been observed in nursery soil newly converted from field crops to conifer seedlings. Pine seedlings having mycorrhizal roots are usually thrifty.

No specific study of mycorrhizae was made in this investigation. Observations are reported only as indications of the need for more facts regarding the interrelations of mineral nutrients, mycorrhizae, and pines.

Apparently the most satisfactory form of phosphatic fertilizer for pine nursery soil is treble superphosphate. Results of this study indi-

cate that in view of effectiveness and cost, 500 pounds per acre is the maximum quantity of treble superphosphate that should be applied. In some cases this rate may prove excessive, stimulating growth to such an extent that tops and roots must be pruned.

Ample justification exists for expecting beneficial results from the application of rock phosphate, although inconclusive results were obtained in these tests. On many plots treated with it a trend toward greater length of roots and height of tops was observed. In the acid soil that is necessary for pine seedlings, rock phosphate should be available. The light texture and organic-matter deficiency of the soils in which these experiments were made are unfavorable to liberation of phosphoric acid from rock phosphate. As the organic-matter supply in the soil increases, yearly additions of rock phosphate should in time augment the total supply to a point at which the annual absorption of phosphorus by the pine seedling crop is currently replaced. Continued addition of large quantities of superphosphate not adjusted to current absorption and loss may result in soil saturation and seedling injury. Chlorosis has been known to result from iron deficiency caused by excess of phosphorus in the soil.

SUMMARY

Exploratory research directed toward better selection and soil management of nursery sites for pines in the Central States was conducted during a 5-year period beginning in 1936. Three Forest Service nurseries were used, those at Chillicothe, Ohio, Vallonia, Ind., and Licking, Mo., which had been established in 1934–36 on land previously used for agriculture. The nurseries at Chillicothe and Vallonia had sandy soils that were alkaline and subject to crusting as a result of concentration of calcium salts from evaporation of soil water or sprinkling water; the Licking nursery had a somewhat heavier silt loam soil that was slightly acid and consequently not subject to this form of crusting. Efforts were made to determine mineral nutrient requirements and deficiencies, and to devise effective methods of amending and fertilizing soils and adjusting soil acidity. The tree species used were shortleaf pine and pitch pine.

Fertilizing tests were started at Chillicothe in the spring of 1937 involving the application of phosphorus, nitrogen, potassium, peat, lime, and combinations thereof. At Vallonia in the fall of 1937 tests were started with 13 treatments including some of the minor elements, soybean meal, phosphorus at three rates of application and at three levels of soil reaction, and potassium. At the three nurseries, tests were started in the spring of 1938 with five phosphatic, four nitrogenous, and three potassic materials together with lime and peat. The 1938 tests at the Licking nursery included a comparison between soil previously occupied by pines and soil previously occupied by hardwoods. At the completion of these experiments tests were set up, in 1939, in which treble superphosphate was applied at seeding time at two different rates per acre and soluble nitrates and soluble potassic salts were applied 2 weeks and 4 weeks, respectively, after seedling emergence. Rock phosphate and greensand marl were further tested in 1939 in conjunction with adjustment of soil reaction by excessive addition of calcium carbonate and by use of dilute sulphuric acid. Results were judged on the basis of seedling density,

height in the first and the second year, and root and top lengths and weights in the second and the third year.

The experiments resulted as follows:

Biotic balance was lacking in soil at the Licking nursery where pines were grown after hardwoods. Where treble superphosphate was applied, increased height growth indicated that it partially compensated for this deficiency. Pines on nearby plots that had been occupied by pines the previous year were uniformly thrifty regardless of whether treble superphosphate had been applied.

Acid-treated peat incorporated in surface soil corrected alkalinity resulting from concentration of calcium salts. In general, peat had a beneficial effect on both density and height.

Increase in soil alkalinity brought about by application of ground limestone resulted in lower seedling density.

Liquid phosphoric acid, treble superphosphate, and iron phosphate increased height growth, root length, and root weight. Aluminum phosphate increased height growth. Height-growth response to these phosphatic fertilizers was more pronounced in soil at pH 4 or 6 than in soil at pH 8. Seedling density varied inversely with quantity of phosphorus applied in these forms. Rock phosphate, applied at the rate of 400 pounds P per acre to soil at pH 4, 6, or 8, did not significantly influence growth or density, although on many plots treated with it a trend toward greater length of roots and height of tops was observed.

Magnesium, potassium, and boron each brought about significantly greater seedling density on Vallonia nursery soil, classifiable as Princeton sandy loam, fall-seeded to shortleaf pine. Potassium sulfate increased density of pitch pine and shortleaf pine seedlings, at Chillicothe and Licking, respectively, measured in the fall of 1938. Copper, manganese, magnesium, zinc, and boron applied at Vallonia all failed under the conditions just mentioned to cause any growth response.

Ammonium sulfate applied 2 and 4 weeks after seedling emergence at the rate of 25 pounds of nitrogen per acre had a favorable effect on length of tops and on weight of roots and tops of shortleaf pine seedlings on Princeton sandy loam. On Huntington silt loam, ammonium sulfate applied 2 weeks after seedling emergence had no perceptible effect on seedling height growth, but the same application 4 weeks after seedling emergence lessened height growth.

No form of potassium tested stimulated height growth of pine seedlings in any of the three nursery soils.

Nitrogen applied at seeding time, in all the forms tested, greatly lowered pine seedling density.